POPULATION GENETICS AND MATING STRUCTURE OF BLUE KING CRAB (*PARALITHODES PLATYPUS*)

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POPULATION GENETICS AND MATING STRUCTURE OF BLUE KING CRAB (*PARALITHODES PLATYPUS*)

A

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Abstract

Blue king crab (*Paralithodes platypus* Brandt, 1850) has been an economically important species in Alaska since the 1970s, but its abundance has decreased substantially since the mid-1980s. Despite Fishery closures, abundances have not rebounded to previous levels. This failure has highlighted the dearth of information on the species and the need for research into genetic population structure and reproductive biology in order to better inform management efforts. Blue king crab tissue and hemolymph samples were collected from eight geographically distinct locations in Southeast Alaska, the Bering Sea, and Russia (n = 770). Allele frequencies at 10 polymorphic microsatellite loci were compared among collection locations. Moderate genetic differences were detected among all locations (overall $\hat{F}_{ST}$ = 0.027, SE = 0.005). Heterogeneity was detected among temporal samples collected at the Pribilof Islands and St. Matthew Island. Comparisons suggested allele frequencies within each location had changed over time. Mating structure was examined by genotyping 20 progeny from each of 44 blue king crab broods collected from 3 different locations in the Bering Sea. All evidence supported single paternity for this species. This study suggests that Alaskan blue king crab stocks be managed at the population level, monitored for temporal genetic changes, and that potential future enhancement activities incorporate the single paternity mating system into determinations of broodstock composition and number.

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Introduction

Historically, blue king crab (*Paralithodes platypus* Brandt, 1850) have supported fisheries throughout their range in the North Pacific including Japan, Russia, and the US (North Pacific Fishery Management Council 2010). Alaskan blue king crab (BKC) fisheries opened in the Bering Sea in the mid-1970s but declined dramatically after peaking in the early 1980s. Though the actual causes of BKC declines are unknown, there are several theories about why these populations crashed that include overfishing (Orensanz et al. 1998), temperature changes causing direct or indirect stressors (Somerton 1985), natural recruitment variation (Zheng & Kruse 2000a, 2006), and past trawling on habitat occupied by mature females (Dew & McConnaughey 2005). Efforts to increase Pribilof Islands BKC population size to previous abundances have failed (Bowers et al. 2011).

The two federally managed stocks of BKC in Alaska are located in the Bering Sea in the vicinity of the Pribilof Islands and St. Matthew Island (North Pacific Fishery Management Council 2010). Recovery of Pribilof Island and St. Matthew Island BKC populations is hindered by lack of basic life history and demographic information for these populations (Stevens et al. 2008b). This lack of information includes reliable estimates of biomass, natural mortality, and recruitment into the fishery. Genetic population structure is also unknown for this species, which further hinders efforts to properly manage BKC fisheries (North Pacific Fishery Management Council 2010).

Genetic studies can provide information on gene flow, effective population size, and genetic divergence (Palsboll 1999). This information is very useful to managers because it can then be used to determine management units, populations which are genetically different enough that each population should be monitored and managed separately (Palsboll et al. 2007). In addition to aiding understanding of population structure, genetic studies can help infer mating structure (Toonen 2004; McKeown & Shaw 2008). Understanding mating structure is particularly important in fisheries stock assessment because reproductive life history parameter estimates can help discern the biological characteristics that shape population structure (Swain et al. 2005). Mating structure is also important if enhancement is used as a management tool because mating structure influences the effective size of a local population and the brood stock used to augment local populations (Sugg & Chesser 1994; Ward 2006). This study was undertaken to determine the population genetic structure and mating structure of BKC.

Distribution, Reproduction, and Development

Blue king crab are patchily distributed from Peter the Great Bay in the Sea of Japan north into the Arctic ocean and in Alaskan waters from the Bering Strait south to Southeast Alaska (Makarov 1962; Somerton 1985; Koblikov et al. 2010). Juveniles and mating adults tend to be found in shallower water (<100 m depths), while non-mating/molting adults tend to be found in water 200 m or deeper (Lysenko 2001). Depth separation between mating and non-mating/molting adults is seasonal and tied to preferences for shallower water during the molting and mating months (Armstrong et al. 1987; Lysenko et al. 2000). In the Bering Sea, BKC occur as deep as 400 m (Makarov 1962).

Blue king crab molt and mate in spring. In the Pribilof Islands, egg extrusion and breeding begins shortly after molting and occurs in April (Somerton & Macintosh 1985; Jensen & Armstrong 1989). Blue king crab molting also occurs in April in the northern portion of the Sea of Okhotsk. In contrast, molting (and presumably mating) occurs in May in the southern portion of the Sea of Okhotsk (Lysenko 2001). On average, BKC females produce a brood every two years. This biennial reproduction cycle is possibly due to physiological and energetic constraints BKC incurred by living in a harsh environment. Lower quality food sources or a reduction in feeding activity due to low water temperatures may make it difficult for all but the smallest BKC females to produce enough somatic and ovarian growth to molt and produce eggs every year (Jensen & Armstrong 1989). It has been hypothesized that this two year cycle may divide BKC populations into two groups that molt and breed in alternate years (Somerton & Macintosh 1985; Lysenko 2001).

The harsh environmental conditions BKC experience also play a role in brooding time of BKC. Blue king crab have a brooding time of approximately 13 months (Somerton & Macintosh 1985; Armstrong et al. 1987; Jensen & Armstrong 1989). However, because colder temperatures slow physiological processes, BKC likely have a longer brooding time in cold water (Stevens 2006a). Laboratory estimates for BKC brooding time vary from approximately13 months for crab collected from the Pribilof Islands and held at 4°C, to an estimated 14 months (hatching began 13 months after collection) for crab collected from Little Diomede Island and held at the ambient water temperature of 3.4 to 8°C (Stevens 2006b; Herter et al. 2011). Lab experiments have also shown hatch

duration significantly increases as both water temperature and holding time in the lab increase (Stevens 2006a; Stevens et al. 2008b; Herter et al. 2011).

After hatching, BKC larvae develop through four zoeal stages before entering their single glaucothoe stage (Hoffman 1968). The zoeal stages are planktonic and last approximately 53 days, during which the larvae exhibit a diel migration to depths of 30-40 meters during the day and 10-20 meters at night (Wainwright et al. 1992). The glaucothoe stage lasts approximately 18 days, before molting into the C1, or first juvenile crab stage (Stevens et al. 2008a). Juvenile crab remain on the ocean floor, eventually maturing into adults.

For the purposes of fisheries management, female BKC reach sexual maturity at approximately five years of age, whereas males mature at approximately six years of age (North Pacific Fishery Management Council 2003). Size at 50% maturity for Pribilof Island female BKC is 96 mm carapace length, whereas St. Matthew Island females reach 50% maturity at 81 mm carapace length. Pribilof Island male size at 50% maturity is 108 mm carapace length and St. Matthew Island males reach this at 77 mm carapace length (Somerton & Macintosh 1983; Otto & Cummiskey 1989).

Divergence and Population Structure

For the purpose of this paper, the terms ‘stocks’ and ‘populations’ refer to two different ways to define groups of BKC. Throughout the literature, definitions of ‘stocks’ and ‘populations’ vary widely and these terms are sometimes used interchangeably. The result is that practical application of these terms can be somewhat arbitrary (Dizon et al.

1992; Balloux & Lugon-Moulin 2002). For this study, ‘population’ and ‘stock’ are not used interchangeably. ‘Population’ refers to an aggregation of individuals caught at a particular site. The term ‘management stock’ is used to describe individuals found within a defined area that are subject to the same commercial and recreational fishing regulations (*i.e.*, occur within the same management unit).

Isolation by distance, genetic drift, and selection are all means by which locally adapted and genetically unique subpopulations may arise (Balloux & Lugon-Moulin 2002). It is important to recognize the genetic boundaries of these subpopulations because their management allows for the preservation of local adaptations that may allow the species as a whole to adapt to changing environmental conditions (Dizon et al. 1992; Ward 2006). Larval dispersal and connectivity homogenize subpopulations, thereby counteracting the effects of isolation, local selective pressures, and drift. The balance between forces that act to differentiate a species and those that act to homogenize a species lead to the genetic population structure of a species (Jones et al. 2007). Understanding genetic connectivity can help by refining boundaries of management units, predicting how management stocks may be affected by changing regulations, refining boundaries of management units, and can help identify areas where conservation efforts may have the greatest benefit (Begg et al. 1999; Bradbury et al. 2008).

Genetic differences among populations will be reduced if adults or juveniles migrate between populations and breed. However, tagging studies of mature male BKC have shown no evidence of adult migrations between the Pribilof Islands and St. Matthew

Island (Otto & Cummiskey 1989), a distance of approximately 360 km. If genetic differences among BKC populations are low, it could be the results of larval dispersal from one population to another. If large genetic differences among populations are present, it may be an indication that BKC larvae tend to settle near the populations in which they were spawned. It should be noted that the time scales for populations to be considered genetically connected versus the time scale of larval dispersal are very different. Populations can be considered genetically connected if several larvae are exchanged among populations each generation. Larval duration time is in the order of months.

Dispersal distance of larvae is influenced by many factors. The time larvae spend in the plankton (planktonic duration) accounts for most of the variability in larval dispersal distance in species that do not exhibit behaviors that cause them to stay close to the ocean floor (Shanks et al. 2003). Physical forces (ocean currents, upwelling, eddies, and gyres) and behavior of the larvae (active swimming, vertical migrations within the water column) influence dispersal distance (Raimondi & Keough 1990; Cowen 2002; Iacchei et al. 2013). Increased knowledge of these forces, as well as larval dispersal patterns, has led to the discovery that some species show significant genetic divergence among locations, despite possessing long larval duration times (Palumbi 1994; Strathmann et al. 2002; Sherman et al. 2008).

Although movement of pelagic zoeal stages of BKC has not been studied, research on the dispersal of snow crab may provide some insights into BKC dispersal. Parada et al.

(2010) developed an individual based model to simulate the dispersal of snow crab larvae hatched throughout the middle and outer domains of the eastern Bering Sea shelf. These two domains are bounded by the 50- and 200- m isobaths and include areas where BKC are found (the Pribilof Islands and St. Matthew Island). Parada et al. (2010) reported substantial retention of snow crab larvae northwest of St. Matthew Island (15% -20% retention) and around the Pribilof Islands (25%-30% retention) and larval transport from Alaskan waters into Siberian waters as well as from the Bering Sea north into the Bering Strait. Southward dispersal of larvae from anywhere in the study area was virtually nonexistent under any of the conditions examined.

Despite differences between BKC and snow crab, models of snow crab larval dispersal may offer insights into larval dispersal patterns of BKC on a broad spatial scale. Differences in pelagic time and preferred larval depths occur between snow crab and BKC and could lead to different dispersal patterns between the two species (Incze et al. 1987; Parada et al. 2010). While differences in pelagic duration time and depth may lead to differences in dispersal patterns, similarities in geographic locations may mean larvae from both species are subjected to similar currents. If this is true, models of snow crab larval dispersal in the Bering Sea could offer insights into broad scale BKC larval dispersal in the same areas.

Models of larval BKC dispersal may help create an understanding of where BKC larvae settle. If these models predict larvae settlement into unsuitable habitat, they may also help to explain low larvae recruitment years. In most crab populations there are both

high and low recruitment years (Zheng & Kruse 2006). If recruitment is consistently low, recruitment limitation may occur. Recruitment limitation occurs when the number of larvae is insufficient to offset natural and fishing mortality and results in a net loss of individuals. Stock enhancement could help to increase the number of juveniles and overcome recruitment limitation (Blankenship & Leber 1995; Bell et al. 2008). However, in order to increase the chances that stock enhancement will be successful and not harm the natural population, genetic population structure, and mating structure need to be known (Toonen 2004; Ward 2006; Bell et al. 2008).

Enhancement

One tool to overcome recruitment limitation is enhancement. "Enhancement", otherwise known as stock enhancement, is the release of hatchery reared juveniles into wild populations in order to increase the supply of juveniles, and thereby overcome problems associated with limited natural recruitment (Blankenship & Leber 1995; Bell et al. 2008). Stock enhancement has been practiced since the mid-19th century, and has gained popularity as fisheries continue to become depleted throughout the world (Lorenzen 2008).

Several crustacean species have been the target of successful enhancement programs. Two of the first examples of crustacean stock enhancement occurred in the mid-1800s, when stock enhancement of the American lobster (*Homarus americanus*) and European lobster (*Homarus gammarus*) began (Moquin-Tandon & Soubeiran 1865, Scattergood 1949). Since then, knowledge about effective rearing and release methods and potential

impacts of hatchery releases on wild populations has improved. Recent studies of hatchery released European lobsters have shown that they can be a significant portion of a fisherman's total catch, each released cohort can support the fishery for up to five years, and hatchery reared females can successfully breed after being released (Addison et al. 1993; Nicosia & Lavalli 1999).

Stock enhancement was investigated in Chesapeake Bay for the blue crab (*Callinectes sapidus*). In 2001, the Blue Crab Research Consortium (BCARC) was established to better understand the biology of blue crab and to develop appropriate technologies and guidelines for responsible enhancement activities (Zohar et al. 2008). One goal of the consortium was to determine if differences between wild and hatchery crab would affect the survival of hatchery crab. The few morphological and behavioral differences observed between wild and hatchery crab could be reduced (Davis et al. 2004). Survival of wild versus hatchery juvenile crab was also reported to be similar in a field experiment (Young et al. 2008). Zohar et al (2008) reported that releases into nursery habitats that were below their carrying capacity resulted in significant enhancement of local management stocks; released hatchery crab grew quickly and were able to contribute to the breeding stock as early as 5 months post-release, and that overall survival was high. Genetic tags were used to monitor released crab and their effects on the natural management stocks (Zohar et al. 2008). These findings show that it may be possible to implement a successful crustacean stock enhancement program if proper techniques are followed and there is sufficient knowledge of the management stock.

Although enhancement of Alaskan BKC stocks has been suggested, the knowledge and technology needed to properly rear BKC in a hatchery environment is not as advanced as that for red king crab. To date studies examining hatchery rearing density of BKC up to 42 days post settlement have been conducted (Daly & Swingle 2013). In addition to determining mating structure, studies determining the long term (after 42 days post settlement) optimum rearing density of BKC as well as optimal release age of hatchery BKC should be conducted in order for a successful BKC enhancement program to occur.

Habitat restoration and improved management practices may also help slow or reverse management stock declines. Unfortunately, management efforts may also be hindered by politics, socioeconomic concerns, or lack of ecological knowledge about the management stock in question (Gewin 2004; Hutchings & Reynolds 2004; Ward 2006). Stock enhancement measures may still be needed to ensure recovery despite improvements in management practices and successful restoration of habitat (Bartley & Bell 2008).

Even if sufficient information is available for the species to be enhanced and proper rearing and release techniques are followed, many questions need to be addressed prior to implementation of a stock enhancement program. For instance, several aspects of the genetics of the management stock in question should be determined, including boundaries or ranges of genetically unique populations, population structure, and mating structure (Toonen 2004; Ward 2006; Bell et al. 2008). Knowing population boundaries, population structure, and mating structure allows managers to collect broodstock that mimics the genetics of the wild management stock.

Mating Structure

The mating system of BKC has not been examined, but crustaceans that have been studied display a wide variety of mating systems (Bilodeau et al. 2005). Some crustaceans, such as ghost shrimp (*Callichirus islagrande*) and the American lobster (*Homarus americanus*), exhibit low levels of polyandry (Bilodeau et al. 2005; Gosselin et al. 2005). However, no evidence of multiple paternity was reported in a mating system study of red king crab, which are closely related to BKC (Vulstek et al. 2013).

Mating structure is important for broodstock selection because mating structure can influence the effective size of a population (Nunney 1993; Ward 2006; Pearse & Anderson 2009). Effective population size can help determine the number of individuals and composition of broodstock that should be used for enhancement measures to be successful or at least not harmful to the natural management stock. While there has been some debate about the topic, several studies have reported that mating systems in which multiple paternity occur have the potential to increase the effective population size as compared to mating systems that exhibit single paternity (Sugg & Chesser 1994; Pearse & Anderson 2009; Lotterhos 2011). Lotterhos (2011) reported that the effect of multiple paternity on effective population size was dependent on generation time, the mean and variance in production of offspring, the mean and number of mates a female had, and the distribution of paternity with a brood.

Enhancement may negatively affect a wild management stock if it causes loss of genetic variation, loss of adaptations, change of management stock composition, or change of

management stock structure, and therefore should be closely monitored (Laikre et al. 2010). In order to reduce potential harm to wild management stocks, broodstock that captures the genetic variability of the wild management stock should be used. Understanding the baseline distribution of genetic variation within and among wild management stocks enables managers to determine ways in which the enhancement measures are influencing or changing the genetics of the enhanced management stock (Blankenship & Leber 1995; Lorenzen 2008).

History of the Fishery

Although BKC have supported fisheries throughout their range, their history in Alaska has been notable for its periods of boom and bust. In Alaska, BKC were first specifically targeted by the Japanese in 1966 in response to both the declining red king crab management stock in Bristol Bay and a general decline in Japan's fishing quotas in US waters (Otto 1989). The US began targeted commercial fisheries for BKC in the 1970s at two locations; the Pribilof Islands and St. Matthew Island (North Pacific Fishery Management Council 2010). Both fisheries peaked in the early 1980s and crashed by the mid-1990s. By 2002, both fisheries had been declared overfished and were closed (Bowers et al. 2011). Rebuilding efforts were put in place shortly after the fisheries closed, but only the St. Matthew Island management stock recovered to the point where a commercial fishery was reopened in 2009 (Bowers et al. 2011). While not considered overfished, the St. Matthew island BKC fishery was closed for the 2013/2014 season because of concerns of low abundance.

Pribilof Island Fishery

The Pribilof Island BKC fishery was once profitable but is now considered overfished. The first US fishery for BKC began in 1973 near the Pribilof Islands (North Pacific Fishery Management Council, 2010). Total catch of BKC in the Pribilof Island fishery peaked in the 1980/1981 season at just under 11 million pounds and peaked economically the next season (1981/1982) when the fishery was worth $13.6 million (Bowers et al. 2008). The management stock crashed shortly thereafter and the fishery remained closed from 1988 through 1995. In 1995, National Marine Fisheries trawl surveys indicated an increase in BKC abundance and a joint red king crab and BKC fishery was opened. The fishery remained open until 1999, when it was closed due to low biomass estimates from trawl surveys and little interest in the fishery (Bowers et al. 2011). The Pribilof Island BKC management stock was declared overfished in 2002 and a rebuilding plan was developed the following year (Zheng & Pengilly 2003).

A decade after a rebuilding plan was put in place, the Pribilof Island BKC management stock has continued to decline. Part of the uncertainty in BKC management has come from biomass estimates that provide little management guidance because they are bounded by huge confidence intervals. In this document, biomass point estimates are reported with the lower bound on the confidence interval (CI) being the number of BKC caught in the specified location during the survey and the upper bound as the biomass point estimate plus the upper 95% CI. Lower bounds are reported as number of crab because the lower 95% CIs obtained from state reports are uninformative (i.e., result in a negative biomass of crab). When the Pribilof Island management stock was declared

overfished in 2002, the total mature male biomass was estimated at 2,019 tons (CI=12 crab to 4,694 tons; Chilton et al. 2011). Despite implementation of a rebuilding plan, the Pribilof Island fishery remains closed due to very low biomass estimates (Bowers et al. 2011). The 2013 estimated mature male biomass was 250 tons (CI =6 crab to 416 tons). This is the fourth lowest estimated biomass in the history of the fishery (Daly et al. 2013).

St. Matthew Island Fishery

A second BKC fishery located near St. Matthew Island has closely followed the general boom- bust cycle seen in the Pribilof Island fishery. Blue king crab were known to inhabit the waters near St. Matthew Island in the 1940s (Otto 1989). However, a directed US fishery was not opened there until 1977. Effort in St. Matthew BKC fishery was sporadic from 1977 to 1981, but after a successful BKC fishery in the Norton Sound district in 1981, effort in the St. Matthew fishing district increased sharply (Bowers et al. 2011). The fishery peaked in 1983 with a harvest of 9.5 million pounds and a total value of $25.8 million (Zheng & Kruse 2000b; Bowers et al. 2011). Harvest levels declined thereafter and culminated in the early closure of the fishery in 1998 (Bowers et al. 2011). Low biomass was estimated based on trawl and pot surveys conducted the following year, which led to the St. Matthew Island BKC management stock being declared overfished in 1999. A rebuilding plan was adopted in 2000 (Zheng & Kruse 2000b).

Unlike the Pribilof Island fishery, rebuilding efforts for the St. Matthew Island management stock have been successful to the extent that it was declared rebuilt in September 2009. A commercial fishery re-opened in October 2009 with a total allowable

catch of 1.05 million pounds. A total of 460,859 pounds of legal BKC were caught by seven vessels that year, resulting in a catch per unit effort (CPUE) of 10 legal crab per pot and an ex-vessel value of $1 million (Bowers et al. 2011).

The "rebuilt" status of the St. Matthew Island BKC management stock does not necessarily mean it has returned to historic levels. A "rebuilt" management stock means that it has met or exceeded the biomass targets specified in the rebuilding plan. Due to a decrease in the number of crab caught at a single station in 2013, the estimated mature male biomass dropped to 2,022 tons (CI = 60 crab to 8,882 tons; Daly et al. 2013). While lower than the 2012 mature male estimated biomass of 5,652 tons (CI = 164 crab to 9,320 tons), it is still nearly twice the estimated mature male biomass in 1999 of 1,302 tons (CI = 67 crab to 1,767 tons) when the fishery was declared overfished (Foy & Armistead 2013).

Despite the marked increase in the St. Matthew Island BKC management stock biomass, actual biomass levels are still uncertain. The confidence intervals for all BKC biomass estimates are very large and translate into great uncertainty in the actual management stock size estimates. After catches in both fisheries peaked in the 1980s, low point biomass estimates have also been problematic. The combination of low biomass estimates and great uncertainty surrounding these estimates has led to periods of low interest in both fisheries as well as periodic, temporary closings of the Pribilof Island fishery before its latest closure in 1999. These problems continue in both fisheries and may be detrimental both to sustainably fishing the St. Matthew Island BKC management

stock and to rebuilding the management stock near the Pribilof Islands. Because of these problems, caution should be used in any decisions where a minimum biomass or management stock size is needed for management purposes.

Current Management

Fisheries managers lack reliable estimates of many basic BKC life history traits and recruitment information that would allow them to estimate important parameters used in their management models. In order to overcome these limitations management models for BKC use information from the historical performance of the fisheries and when necessary, information from other species (North Pacific Fishery Management Council 2011). To better manage BKC management stocks, it is essential for more information on life history parameters and stock structure to be incorporated into management models. Models which better describe BKC management stock dynamics have the potential to help avoid future BKC management stock crashes in the St. Matthew Island fishery as well as to aid recovery efforts for the Pribilof Island management stock. Population genetic data can also help managers to define population structure and to define management units.

Oceanography

Blue king crab inhabit a large geographic area and are subject to many different oceanographic conditions throughout their range. The southeastern end of their range is characterized by extensive fjord complexes, freshwater input from glacier melt, alternating deep basins, shallow stretches, and bays (Weingartner et al. 2009). The

middle of their range spans the Bering Sea, which is subject to several currents of varying velocities and smaller scale eddies and gyres (Stabeno & Reed 1994). Western portions of the range of BKC are found within the Sea of Okhotsk, which is partially isolated from the waters of the Bering Sea via the Kuril Islands. Waters within the Sea of Okhotsk come primarily from the Bering Sea, but warm water from the Sea of Japan also enters the Sea of Okhotsk (Talley 2001). Understanding the oceanography of the areas BKC inhabit is important to understanding larval dispersal throughout the species' range as well factors that influence crab throughout their life cycle.

Ocean circulation throughout Southeast Alaska is affected by many forces and can vary both seasonally and between adjacent bays or fjords. Circulation of interior waters (waters within the Southeast Alaska Archipelago) is generally northward, with strong winter winds contributing to downwelling. These forces may help to transport heat, freshwater, and organisms north to Southeast Alaska from the Pacific Northwest (Weingartner et al. 2009). The steep, narrow channels of Southeast Alaska are also influential in determining water circulation. A narrow shelf region, coupled with the deep passageways present in the interior waters of Southeast Alaska, may mean that offshore waters can easily mix with inshore waters in some areas (Weingartner et al. 2009). Offshore waters move north via the Alaska Stream and turn west in the Gulf of Alaska until they reach the Aleutian Islands (Reed & Stabeno 1996; Stabeno et al. 2004). Mean current flow of outside waters in Southeast Alaska and west to the Aleutian Islands is shown in Figure 1.

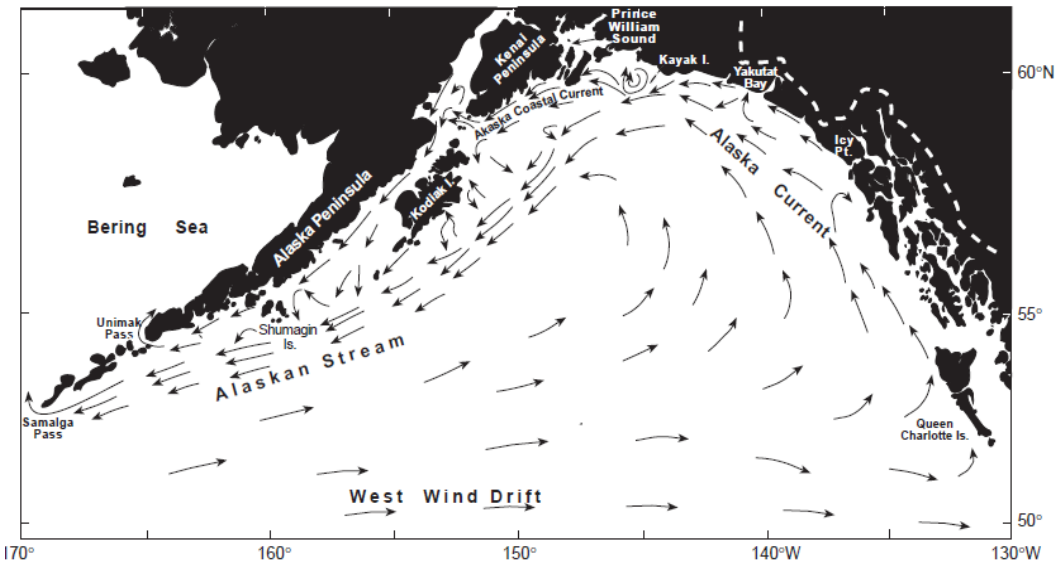

Figure 1: Mean water flow in the North Pacific Ocean. (From Stabeno et al., 2004.)

Water from the Alaska Stream flows west from the Gulf of Alaska and then through one of several passes in the Aleutian Islands, most prominently Amchitka Pass (Figure 2). From there, the water flows into the Bering Sea. Once in the Bering Sea, this current turns east and forms the Aleutian North Slope Current (Reed & Stabeno 1999). The Bering Slope Current branches off and begins to flow westward, while the Aleutian North Slope Current continues in a generally northeast direction until it exits the Bering Sea via the Bering Strait (Reed & Stabeno 1996). Both currents are characterized by eddies and often by weak, variable flow (Stabeno et al. 1999).

The Bering Slope Current flows into the Kamchatka Current, which flows south and eventually exits the Bering Sea via Kamchatka Pass. The Kamchatka Current is the main current in the western portion of the Bering Sea and has the potential to be a swift current; but due to eddies and variability in flow, its mean velocity is less than that of the

Alaska Stream (Stabeno & Reed 1994; Stabeno et al. 1999). The Kamchatka Current carries waters from the Bering Sea into the Sea of Okhotsk.

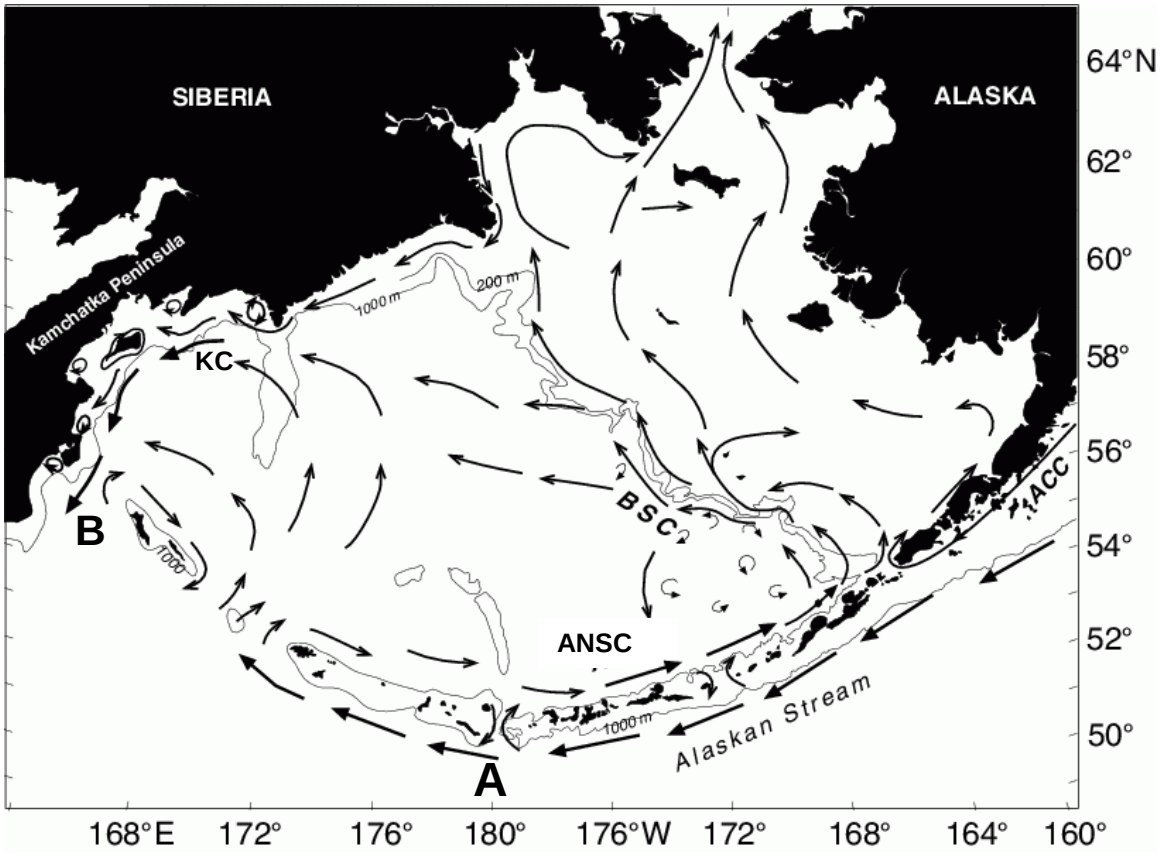

Figure 2: Schematic of mean circulation in the upper 40 m over the Bering Sea basin and shelf. Amchitka Pass is denoted on the map by an 'A', Kamchatka Pass is denoted on the map by a 'B'. The Bering Slope Current is abbreviated 'BSC', the Aleutian North Slope Current is abbreviated by 'ANSC' and the Kamchatka Current is abbreviated KC. (Modified from Stabeno et al., 1999).

As it flows south out of the Bering Sea, the Kamchatka Current exchanges water with the Sea of Okhotsk at the Kuril Islands. Water from the Sea of Japan is also introduced to the Sea of Okhotsk through Soya Strait in the south (Lapko & Radchenko 2000; Talley 2001). The mean circulation of water in the Sea of Okhotsk is counterclockwise, a

product of many of the same forces at work in both the Bering Sea and Southeast Alaska (Figure 3; Talley 2001). After entering the Sea of Okhotsk, the northern flowing West Kamchatka Current carries water into Shelikhov Gulf. The Yamkoy Upwelling is located at the mouth of Shelikhov Gulf, making this area very productive. A large gyre dominates the western portion of Shelikhov Gulf itself, but water can leave the gulf via the Yamskoe Current (Lapko & Radchenko 2000). Once out of Shelikhov Gulf, water flows in a counter clockwise direction until eventually leaving the Sea of Okhotsk and flowing back into the Bering Sea around the southern portion of the Kuril Islands (Talley 2001). These diverse patterns of currents have the potential to cause complicated patterns of larvae dispersal in BKC and may make predicting their impact on among population gene flow difficult.

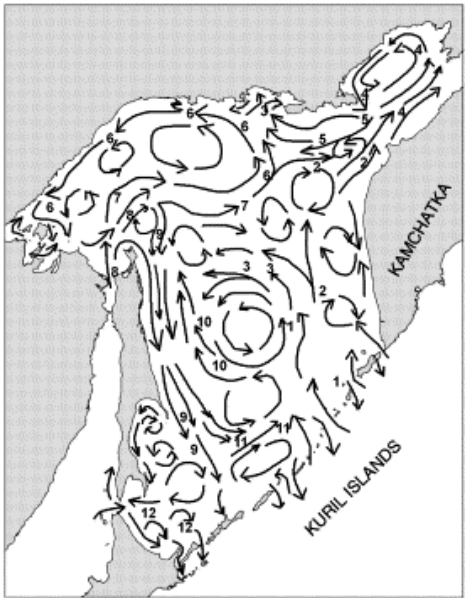

Figure 3: Scheme of general water circulation in active layer in the Sea of Okhotsk in summer. 1: West Kamchatka Current; 2: its Northern Branch; 3: Middle Current; 4: Penzhinskoe Current; 5: Yamskoe Current; 6: Northern Okhotsk Current; 7: Northern Okhotsk Concurrent; 8: Amurskoe Current; 9: East Sakhalin Current; 10: East Sakhalin's Concurrent; 11: Northeastern Current; 12: Soya Current. (From Lapko & Radchenko, 2000).

Current Project

This study addresses three specific questions about genetic population structure and mating structure of BKC: (1) What is the genetic population structure of geographically separated BKC populations? (2) Do changes in genetic population structure occur over time within two BKC populations in Alaska? and (3) How many males does a female BKC mate with within a given mating season? Understanding genetic population structure and how it changes over time is important for predicting responses of populations to future climate and anthropogenic events, and can provide insights into the spatial scale at which BKC management stocks should be managed. Knowing the number of males with which each female BKC mates will help determine the number of broodstock needed if enhancement is used to augment local BKC populations. The overarching goal of this research is to use genetic tools to obtain information that can be used to improve management of BKC stocks throughout Alaska and improve chances for recovery of this commercially valuable species.

Materials and Methods

Sample Collection

Blue king crab tissue and hemolymph samples were collected from eight locations throughout the species' range including Southeast Alaska, the Pribilof Islands, St. Matthew Island, Little Diomede, Chaunskaya Bay, the western Bering Sea, and two locations from Shelikov Gulf in the Sea of Okhotsk (Table 1). Hemolymph samples from the Pribilof Islands and St. Matthew Island were collected by the National Oceanic and Atmospheric Administration during trawl surveys between 2009 and 2011. Blue king crab samples from Little Diomede and tissues samples from the Pribilof Islands and St. Matthew Island (collected between 2006 and 2011) were collected by the Alaska Department of Fish and Game (ADF&G) during pot surveys. Historic BKC tissue samples (1993-1996) from the Pribilof Islands and St. Matthew Island were obtained from ADF&G's Gene Conservation Laboratory (Anchorage, AK). Blue king crab samples from all Russian locations were collected by contributor Vlad Brykov and provided as extracted DNA in 70% ETOH (Figure 4).

Table 1: Location, sample size, type, study, and year of collection for all blue king crab samples.

Location	Sample Size	Sample Type	Study	Year of Collection
(1) Little Diomede	18	Tissue	Spatial & Paternity	2008
(2) Pribilof Islands	236	Tissue & hemolymph	Spatial & Paternity	1993, 1996, 2006, 2009 – 2011
(3) St. Matthew Island	305	Tissue & hemolymph	Spatial & Paternity	1993, 2009-2010
(4) South Shelikhov Gulf	38	DNA	Spatial	1998
(5) North Shelikhov Gulf	39	DNA	Spatial	1999
(6) Western Bering Sea	49	DNA	Spatial	2001
(7) Chaunskaya Bay	50	DNA	Spatial	1999
(8) Southeast Alaska	38	Tissue	Spatial	2011

Location numbers correspond to numbers in Figure 4.

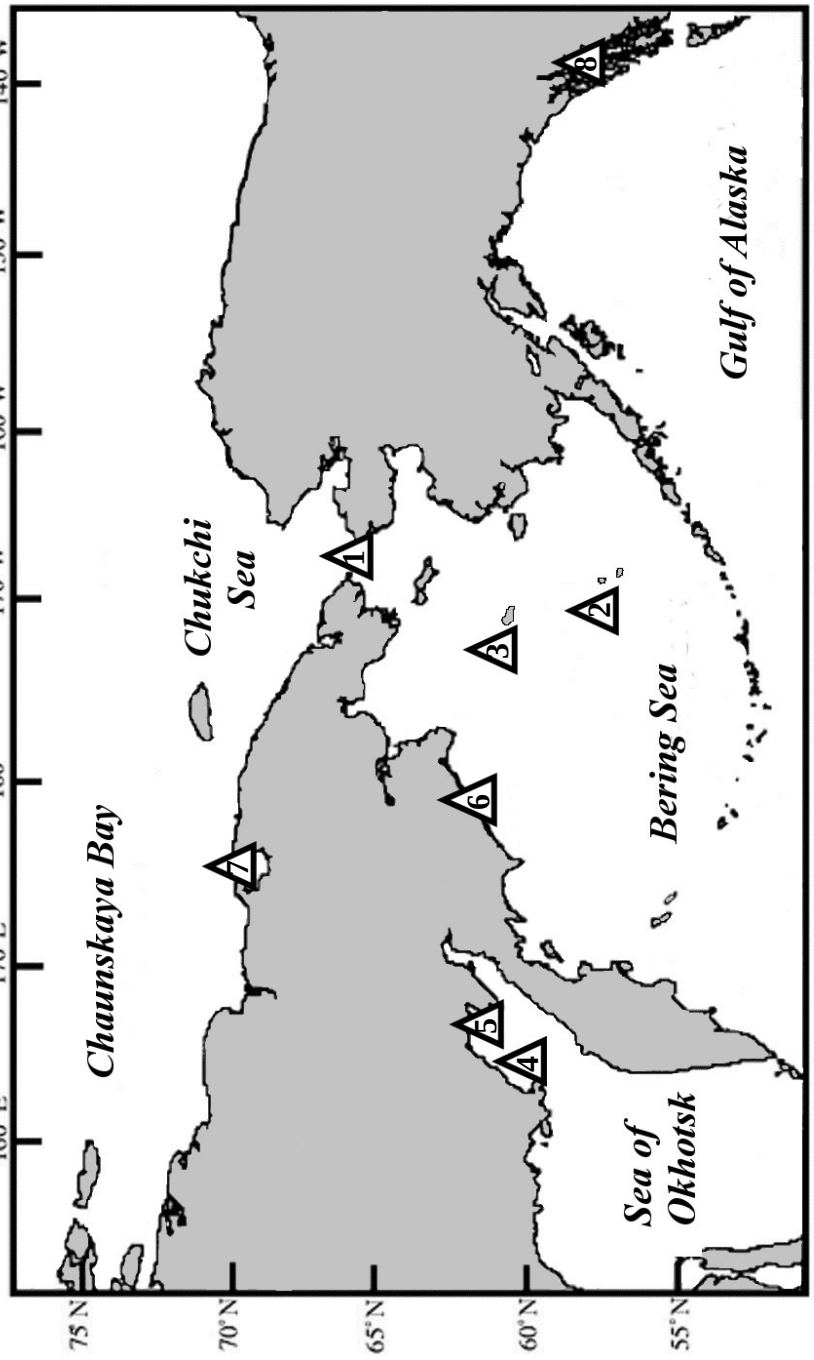

Figure 4: Collection locations of blue king crab samples. For sample size by location and year, see Table 1.

Southeast Alaska samples were taken from BKC caught as bycatch during the 2011 red king crab commercial fishery. Local fisherman delivered BKC to canning facilities in Juneau, Alaska, where the crab were kept in a holding tank until sampling. Samples from Southeast Alaska consisted of a mix of hemolymph and tissue samples. Southeast Alaskan samples were from three separate locations (Lynn Canal, near Douglas Island, and between Juneau and Ketchikan). However, all crab were placed in the same holding tank before sampling began, making it impossible to assign individual crabs to specific areas. Southeast Alaska samples were therefore pooled for all analysis.

Hemolymph samples consisted of no more than 0.2 ml of BKC hemolymph extracted with a sterile syringe and stored in 95% ethanol in a 96 well plate or 1.5 ml centrifuge tube. If tissue was collected, a small amount of tissue was extracted from the crab and placed in a tube containing 95% ethanol for storage. Both hemolymph and tissue DNA samples were included in the spatial population genetic analysis.

Paternity analyses were conducted on gravid females obtained during ADFG pot surveys around the Pribilof Islands in 2006, Little Diomede in 2008, and St. Matthew Island in 2009-2011. All crab were brought to Alutiiq Pride Shellfish Hatchery in Seward where tissue samples were taken from each female and embryo and/or zoeal stage 1 larvae (Z1) samples were taken from each brood and preserved in 95% ethanol. Twenty progeny from each female were haphazardly sampled and genotyped for the paternity study.

Microsatellite Genotyping

Species specific microsatellite primers were developed for this study. Extracted DNA from 30 BKC samples were sent to the University of Georgia Savannah River Ecology Lab to develop microsatellite primers. Twenty three BKC specific microsatellite loci were developed (Appendix B) (Stoutamore et al. 2012). Ten of these loci were chosen to examine genetic population structure based on heterozygosity, number of alleles per locus, lack of null alleles, and ease of scoring alleles at the chosen loci.

Protocols modified from Ivanova et al. (2006) were used to extract genomic DNA from hemolymph samples. Tissue and all progeny samples were subjected to a proteinase K and ammonium acetate precipitation technique to extract genomic DNA. A standard salt precipitation method was used to separate Russian DNA samples from the 70% ethanol in which they were shipped. Both methods were modified from Sambrook (2001). All DNA was then stored at -20°C until analyzed.

In order to amplify the desired DNA sequences, polymerase chain reactions (PCR) were used. Polymerase chain reactions had a final volume of 10 µl and final concentration of 1x PCR buffer, 1.4 to 2.0 mM $MgCl_2$, 0.1 mg/ml BSA, 0.6 to 0.8 µM dNTPs, 0.3 to 0.8 µM forward primer, 0.03 to 0.08 µM forward labeled primer, 0.3 to 0.8 µM reverse primer, 0.5 units of Taq and 2 µL of DNA (5ng/µL) per reaction. PCR buffer consisted of 25 mM $MgCl_2$, 1 M KCl, and 1 M Tris-HCl (pH 9.0). Forward primers that had been fluorescently labeled with IRDye ® infrared dye (LI-COR, Lincoln, NE) were included in the PCR. A DNA Engine Tetrad PTC-225 Peltier thermal cycler (Bio-Rad

Laboratories, Hercules, CA) was used to amplify the microsatellite loci. PCR product (5 µL) was mixed with 0.5 µL to 1.0 µL of formamide running buffer. A 0.25 mm thick 6.5% polyacrylamide gel (19:1 acrylamide: bisacrylamide) was then used as a medium to amplify 1µL of the PCR product/running buffer solution per lane. Li-Cor size standard (50-350 base pairs) was added every 12 lanes. The amplified product was visualized on a Li-Cor 4300 DNA analyzer, with microsatellite sizes determined using Saga Generation 2 microsatellite analysis software (LI-COR, Lincoln, NE).

Genetic Diversity and Genetic Population Structure Analysis

Genetic diversity and genetic divergence measures were estimated with several programs. The program GENEPOP v.4.1.10 (Rousset 2008) was used to perform pseudo-exact tests for departure from Hardy-Weinberg expectations at each locus and to estimate gametic disequilibrium between all pairs of loci in each collection. GENEPOP was also used to perform homogeneity tests (*G*-tests) among sample locations and to estimate allele frequencies. Fisher's pseudo-exact tests were calculated with GENEPOP to test if BKC spawning in even years are genetically distinct from BKC spawning in odd years. Probabilities were calculated by locus across all populations. The program FSTAT 2.9.3.2 (Goudet 2002) was used to estimate Wright's *F* – statistics, pairwise $\hat{F}_{ST}$ and genetic diversity measures. Estimates of $\hat{F}_{ST}$were estimated by Weir and Cockerham's θ (1984) and 95% confidence intervals of multilocus mean $\hat{F}_{ST}$ estimates were estimated by bootstrapping samples across all loci (Goudet 2002). Wright's *F* – statistics were calculated by population and across all loci. Mean observed heterozygosity ($\overline{H}_O$) and mean expected within subpopulation heterozygosity ($\overline{H}_S$) were estimated using the

hierfstat package in R. The program SMOGD (Crawford 2010) was used to estimate overall and pairwise Jost's $\overline{D}$ (2008), as well as overall Hedrick's $\overline{G}'_{ST}$. Significance levels were adjusted for multiple tests with a sequential Bonferroni correction where appropriate (Rice 1989). Using Microsoft Excel Office 2007, hierarchical analysis using *G*-tests were conducted to determine whether genetic differences exist at various spatial scales or between temporally separated samples collected from the same location (Sokal & Rohlf 2011). In order to better visualize the relationships among the multivariate data, allele frequencies were arcsine square root transformed and principal component analysis (PCA) was performed using the *adegent* R package (Jombart et al. 2008).

Spatial Analysis of Molecular Variance (SAMOVA) and Arelquin were also used to investigate spatial population structure of BKC. The program SAMOVA v. 1.0 uses a simulated annealing procedure to define groups of populations that are maximally differentiated from each other as well as geographically homogenous (Dupanloup et al. 2002). This approach eliminates the need to assign groups *a priori* when testing genetic structure. Populations were assigned to K different groups, with groupings of K = 2 through K= 7 tested. The annealing process was repeated 100 times for each K. Each grouping was then tested for statistical significance in Arelquin v. 3.5.1.2 (Excoffier & Lischer 2010) using 10,000 permutations. The significant grouping that maximized variance attributed to differences among groups of populations (F_{CT}) was chosen as the best grouping (Dupanloup et al. 2002).

Bottleneck Analysis

The program BOTTLENECK v.1.2.02 (Cornuet & Luikart 1996) was used to test for recent, large decreases in abundance of BKC. A severe bottleneck produces a loss of both heterozygosity and allelic diversity as compared to what would be expected were the population at mutation-drift equilibrium (Cornuet & Luikart 1996). To test for evidence of a population bottleneck, this program compares the expected heterozygosity (based on the number of alleles present and population size and assuming mutation – drift equilibrium) to the expected heterozygosity as calculated by Nei's gene diversity (Nei et al. 1975, Cornuet & Luikart 1996). A two phase model (TPM), which is a combination of a stepwise mutational model (SMM) and an infinite alleles model (IAM), was used to test for evidence of recent bottlenecks in both temporally sampled locations (St. Matthew Island and the Pribilof Islands). Three proportions of SMM in the TPM were used (70%, 80%, and 90%) and 10,000 iterations run for each proportion. Within the BOTTLENECK program, the variance of mutations larger than one step in the TPM can be adjusted. We kept the default variance of 12.00 for each proportion tested. A Wilcoxon signed-rank test was used to test for excess heterozygosity.

Mating System Analysis

Twenty progeny were sampled from each of 44 brooding females collected from the Pribilof Islands (n = 9), St. Matthew Island (n = 17), and Little Diomede (n = 18). These progeny and their mothers were genotyped at five microsatellite loci (L21, L40, L19, L39 and L31) to determine if BKC exhibit single or multiple paternity. Alleles present in

each brood were compared to maternal alleles. Multiple paternity was indicated if three or more non-maternal alleles were present.

The power to detect multiple paternity within a single brood was determined with the program PrDM v.1 (Neff & Pitcher 2002). Using a Monte Carlo simulation method, PrDM determines the probability that multiple mating could be detected based on the number of loci used, the number of alleles at each locus, allele frequencies in each population examined, the number of potential sires, and the relative reproductive contributions of each sire. Simulations were conducted by using population allele frequencies determined using five loci, twenty progeny per brood, two or three potential sires, and the allele frequencies determined in the population structure analysis. Relative reproductive contributions of 1:1 and 9:1 were used in simulations with two potential sires, and relative reproductive contributions of 1:1:1 and 3:2:1 were used in simulations with three potential sires.

Results

Genetic Diversity

Mean expected within-population heterozygosity ($\bar{H}_S$) and mean allelic richness ($\bar{A}_R$) varied across the study area; the lowest values were found in Southeast (Table 2). Mean within population $\bar{H}_s$ ranged from 0.594 (SE = 0.016) to 0.729 (SE = 0.012). Mean allelic richness ($\bar{A}_R$) within each population ranged from 5.299 (SE = 0.186) to 7.300 (SE = 0.254). Mean within population $\bar{F}_{IS}$ ranged from -0.039 (SE = 0.004) to 0.036 (SE = 0.007).

Some departures from Hardy-Weinberg expectations and gametic disequilibrium were observed. Departures from Hardy-Weinberg expectations were observed in 19 of 100 tests, but only four of the departures remained significant after Bonferroni corrections. No consistent patterns among loci or populations were observed for these four significant departures. Homogeneity tests indicated significant differences between the two Shelikhov Gulf sites which lead to the sites being kept separate for the rest of the analysis. While variation of inbreeding coefficients ($\bar{F}_{is}$) did occur across the study area, none of the $\bar{F}_{is}$ estimates were statistically significant, and ranged only from $\bar{F}_{is}$ = -0.039 (SE = 0.004) to $\bar{F}_{is}$ = 0.036 (SE = 0.006). There was little evidence of non-random mating or high frequencies of null alleles at any of the sampled locations. As a result of these analyses, all 10 of the loci examined for departures from Hardy-Weinberg proportions, linkage disequilibrium and large $\bar{F}_{is}$ values were included in further analyses to examine BKC population genetic structure.

Table 2: Mean expected ($\overline{H}_S$) and observed ($\overline{H}_O$) heterozygosities, inbreeding coefficient (F_{IS}), and mean allelic richness ($\overline{A}_R$).

Population	$\overline{H}_S$	$\overline{H}_O$	$\overline{F}_{IS}$	$\overline{A}_R$
(1) LD	0.677 (0.016)	0.678 (0.016)	0.000 (0.016)	7.300 (0.254)
(2) 9396_PI	0.620 (0.020)	0.664 (0.021)	-0.036 (0.006)	6.741 (0.230)
(2) PI	0.684 (0.014)	0.588 (0.017)	0.036 (0.007)	6.767 (0.227)
(3) 93_SMI	0.651 (0.017)	0.635 (0.019)	-0.039 (0.004)	6.466 (0.238)
(3) SMI	0.684 (0.015)	0.568 (0.016)	0.021 (0.007)	6.783 (0.218)
(4) SSG	0.725 (0.013)	0.687 (0.017)	0.004 (0.013)	6.334 (0.239)
(5) NSG	0.724 (0.012)	0.687 (0.016)	0.033 (0.016)	6.426 (0.188)
(6) WBS	0.729 (0.012)	0.708 (0.015)	-0.011 (0.007)	6.676 (0.212)
(7) CSB	0.683 (0.019)	0.680 (0.023)	-0.029 (0.011)	6.505 (0.227)
(8) SEAK	0.593 (0.016)	0.579 (0.019)	-0.002 (0.011)	5.299 (0.186)

Heterozygosities, allelic richness and inbreeding coefficients are calculated within populations and across all loci. Standard errors are in parenthesis. Populations: 9396_PI (Pribilof Island samples collected in 1993 and 1996), 93_SMI (St. Matthew Island samples collected in 1993), SSG (South Shelikhov Gulf), NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay) LD (Little Diomede), PI (Pribilof Islands), SEAK (Southeast Alaska), SMI (St. Matthew Island), and WBS (Western Bering Sea). Numbers appearing before the population abbreviation refer to population locations in Figure 4.

Geographic Population Structure

Genetic population structure was observed at the spatial level of our sampled populations. After correction for multiple tests, all pairwise homogeneity tests for genetic differentiation among geographically separated populations were significant, suggesting significant differences among most of the sampled locations. Pairwise $\hat{F}_{ST}$ tests showed similar results (Table 3). Population differentiation as indicated by Wright's F_{ST} was moderate ($\hat{F}_{ST}$ = 0.027, SE = 0.005), but supported by mean overall Jost's $\overline{D}$ = 0.0907 (SE = 0.007), and mean overall Hedrick's $\overline{G'}_{ST}$ of = 0.1197 (SE = 0.008).

Similarly, PCA separated sample locations along the first two principal components into a geographically coherent picture, indicating genetic differences occur among locations across the study area (Figure 5). The first two principal components accounted for 35.29% and 16.83% of the variation in allele frequencies, respectively. The collections generally fell out in the PCA as would be expected based upon their distribution from east to west across the range of BKC in Alaska and Russia, with Southeast Alaska and Sea of Okhotsk collections showing the greatest divergence from one another. Intermediate to these collections were all of the collections from the Bering Sea (western Bering Sea, Pribilof Islands, St. Matthew Island, Little Diomede) and the Arctic Ocean (Chaunskaya Bay). These Bering Sea populations show some divergence in allele frequencies along PC1 and PC2, but not as much as did the Southeast Alaska and Sea of Okhotsk populations. Despite large geographic separation of Chaunskaya Bay from the rest of the sampling locations, this population clustered with the eastern Bering Sea collections.

Table 3: Pairwise $\widehat{F}_{ST}$, Jost's ($\overline{D}$) values, and *P*-values forspatial homogeneity test of blue king crab samples.

Population	SSG	NSG	CSB	LD	PI	SEAK	SMI	WBS
SSG	-	0.0022	*	*	*	*	*	*
NSG	**0.0045**/ 0.0007	-	*	*	*	*	*	*
CSB	**0.0436**/ 0.0166	**0.0381**/ 0.0166	-	*	*	*	*	*
LD	**0.0375**/ 0.0417	**0.0296**/ 0.0558	**0.0390**/ 0.0522	-	*	*	*	*
PI	**0.0522**/ 0.0749	**0.0422**/ 0.0407	**0.0255**/ 0.0301	**0.0234**/ 0.0240	-	*	*	*
SEAK	**0.0954**/ 0.1015	**0.0894**/ 0.0918	**0.0792**/ 0.1028	**0.0556**/ 0.0287	**0.0376**/ 0.0262	-	*	*
SMI	**0.0375**/ 0.0489	**0.0322**/ 0.0391	**0.0217**/ 0.0313	**0.0164**/ 0.0110	**0.0057**/ 0.0052	**0.0476**/ 0.0296	-	*
WBS	**0.0209**/ 0.0282	**0.0112**/ 0.0139	**0.0187**/ 0.00079	**0.0142**/ 0.0150	**0.0223**/ 0.0241	**0.0619**/ 0.0628	**0.0138**/ 0.0148	-

Pairwise $\widehat{F}_{ST}$ (bold) and Jost's ($\overline{D}$) values are given in the lower diagonal, *P* – values for spatial homogeneity estimates are given in the upper diagonal. '*' indicates a *P* – value of $P < 10^{-5}$. Locations: SSG (South Shelikhov Gulf), NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay), LD (Little Diomede), PI (Pribilof Islands), SEAK (Southeast Alaska), SMI (St. Matthews Island), WBS (Western Bering Sea).

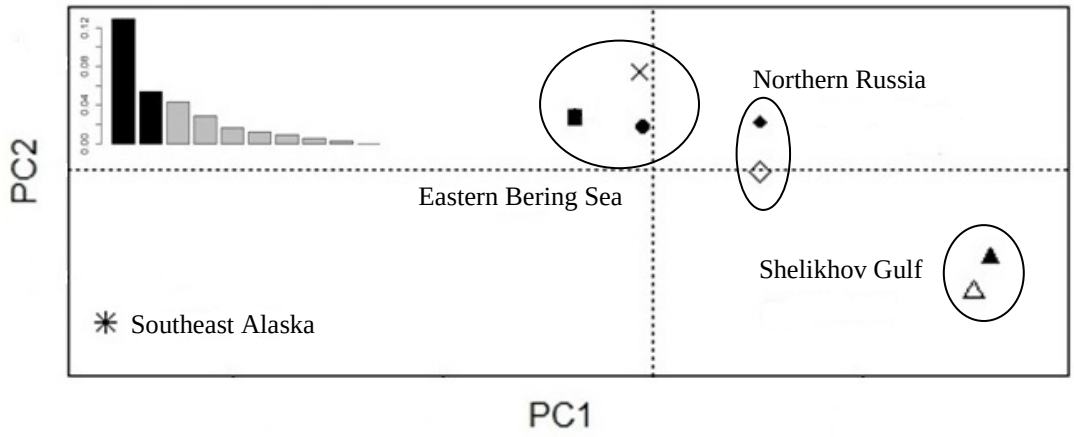

Figure 5: Principal component analysis of blue king crab allele frequency data. Samples include Southeast Alaska (*), St. Matthew Island (●), the Pribilof Islands 2006-2011 (■), Little Diomede (X), Western Bering Sea (◊), Chaunskaya Bay (♦), South Shelikhov Gulf (▲), and North Shelikhov Gulf (△). The inset graph shows the proportion of variance described by the first ten principal components. The black bars represent the two principal components used in the analysis, which combined account for 52.12% of the observed variance.

Although most of the geographically sampled locations differed significantly from each other genetically, Southeast Alaska showed the highest amount of genetic divergence from other sampled populations. The highest $\hat{F}_{ST}$ and $\overline{D}$ estimates in pairwise homogeneity tests were between Southeast Alaska and other populations. Southeast Alaska also showed the most separation in the PCA, primarily along PC1. Similarly, when eastern Bering Sea populations (Little Diomede, St. Matthew Island and the Pribilof Islands) were pooled, hierarchical analysis indicated significant differences in heterogeneity between Southeast Alaska and these pooled locations ($P = 4.112 \times 10^{-6}$). Southeast Alaska was also the first collection to be separated from other sampled locations in the SAMOVA (Table 4). The first population to be separated in SAMOVA represents the population with the highest proportion of among group variation.

Table 4: Spatial analysis of molecular variance (SAMOVA) groupings for K=2 to 7 of blue king crab samples.

K	Groupings	F_{SC}	F_{CT}	P
2	(SEAK) and (all others)	0.01914	0.03812	0.124 ± 0.003
3	(SEAK), (SSG, NSG), (CSB, WBS, LD, SMI, PI)	0.01141	0.03194	0.007 ± 0.001
4	(SEAK), (SSG), (NSG), (CSB, WBS, LD, SMI, PI)	0.01194	0.03001	0.0178 ± 0.001
5	(SEAK), (SSG), (NSG), (CSB), (WBS, LD, SMI, PI)	0.00825	0.02826	0.026 ± 0.002
6	(SEAK), (SSG), (NSG), (CSB), (WBS) (LD, SMI, PI)	0.00552	0.02672	0.036 ± 0.002
7	(SEAK), (SSG), (NSG), (CSB), (WBS), (LD), (SMI, PI)	0.00318	0.02803	0.034 ± 0.002

SAMOVA groupings for K = 2 – 7, variance among groups of populations (F_{CT}) and differentiation among populations within groups (F_{SC}). Locations: SEAK (Southeast Alaska), SSG (South Shelikhov Gulf) and NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay), WBS (Western Bering Sea), LD (Little Diomede), SMI (St. Matthew Island), PI (Pribilof Island).

The Pribilof Islands and St. Matthew Island showed the least genetic divergence of those sites that were statistically significantly different from each other. Pairwise homogeneity tests between these two populations resulted in $\hat{F}_{ST}$ (0.0057) and $\overline{D}$ (0.0052) values well below those of any other significant pairwise comparisons. Although pairwise comparisons suggested statistically significantly differences between St. Matthew Island and the Pribilof Islands, not all statistical tests indicated significant differences. For example, the hierarchical analysis between these two populations resulted in a non-significant P – value (P = 0.750).

Temporal Population Structure

Temporal genetic changes were observed among all of the Pribilof Islands and St. Matthew Island samples. All pairwise homogeneity tests for temporally separated collections were significant after corrections for multiple tests. Pairwise $\hat{F}_{ST}$ tests had similar results (Table 5). This suggests allele frequencies have changed over time in these populations.

Table 5: Pairwise ($\hat{F}_{ST}$), Jost's ($\bar{D}$) values, and *P*-values for temporal homogeneity test of blue king crab samples.

Population	93_PI	93_SMI	96_PI	PI	SMI
93_PI	-	0.0130	*	*	*
93_SMI	**0.0091**/ 0.0050	-	*	*	*
96_PI	**0.0072/** 0.0036	**0.0117/** 0.0081	-	*	*
PI	**0.0050/** 0.0051	**0.0093/** 0.0115	**0.0065/** 0.0057	-	*
SMI	**0.0038/** 0.0023	**0.0073/** 0.0036	**0.0255/** 0.0060	**0.0057/** 0.0052	-

Pairwise $\hat{F}_{ST}$ (bold) and Jost's ($\bar{D}$) values are given in the lower diagonal, *P* – values for homogeneity estimates are given in the upper diagonal. '*' indicates a *P* – value of $P < 10^{-5}$. Locations: 93_PI (Pribilof Island samples collected in 1993), 93_SMI (St. Matthews Island samples collected in 1993), 96_PI (Pribilof Island samples collected in 1996), PI (Pribilof Islands samples collected between 2006 and 2011), SMI (St. Matthews Island samples collected between 2009 and 2011).

Hierarchical analysis also indicated significant heterogeneity among temporal samples ($P = 1.596 \times 10^{-168}$), giving further evidence that changes in allele frequencies occurred at St. Matthew Island and the Pribilof Islands between sampling dates in the 1990s and those in the 2000s.

Population bottlenecks could account for the rapid changes in allele frequencies observed in the temporal study, but tests for excess heterozygosity suggest no recent population bottlenecks have occurred at either St. Matthew Island or the Pribilof Islands. After a bottleneck occurs, allelic richness decreases rapidly and results in a greater heterozygosity than expected from the number of alleles observed within the population (Cornuet & Luikart 1996). Wilcoxon tests for all mutation models (e.g. 70%, 80% or 90% SMM) were non-significant for heterozygosity excess ($P > 0.999$), indicating no recent bottleneck has occurred at either St. Matthew Island or the Pribilof Islands. Similar estimates of allelic richness were observed between temporal collections within each location, further suggesting that no recent bottlenecks have occurred.

There was no evidence to support the presence of two genetically distinct cohorts that breed in alternate years at either the Pribilof Islands or St. Matthew Island. While sample sizes for odd and even years at each site were small, Fisher's pseudo-exact tests calculate an exact probability value for the relationship between variables and are therefore very useful for small data sets. When sites were pooled, Fisher's pseudo-exact tests for a difference in allele frequencies between years were significant at a single locus (L39, $P = 0.01582$). Therefore, nine of ten loci tested showed no significant differences in allele frequencies between crab that mate in even years and those that mate in odd years. *P*-values for the remaining nine loci ranged from $P = 0.05681$ to $P = 0.96724$.

Mating Structure

Strong evidence for single paternity in BKC was observed in all broods tested. None of the 44 broods indicated more than two non-maternal alleles at any of the 5 loci examined (Table 6). Genotyping 20 progeny from each of 44 broods at 5 loci provided a high probability of detecting multiple paternity if it had been present. The overall probability of detecting multiple paternity across all broods tested was nearly 100% for each of the paternity models examined. All of the genetic data support single paternity as the mating system of BKC.

Table 6: Alleles detected in each blue king crab brood at each of five microsatellite loci.

Location	Crab/ Brood #	L21	L40	L19	L39	L31
Little Diomede	1	109, **121, 129**, 133	**112, 120**	**102**	**212,** 216, **220**	**183,** 187, **207**
Little Diomede	2	98, **102, 125,** 129	112, **116, 120**	**102, 114**	**208, 216,** 220	**183, 207,** 215
Little Diomede	3	**121,** 129	**112, 116**	**102**	**204,** 212, 220	**175, 179,** 199, 203
Little Diomede	4	109, **125, 129**	**112,** 116, **120**	**102**	**216, 228**	**183, 187,** 199, 207
Little Diomede	5	113, **125, 129**	**108, 112,** 120	**102**	208, **216,** 220	**179, 183,** 199, 207
Little Diomede	6	**113, 121,** 133	**108, 120,** 124	**102,** 110	212, **216,** 220, **224**	**183, 187,** 223
Little Diomede	7	**121, 125,** 129	**120**	**102, 118**	220, **224, 232**	**183,** 199, 203, **207**
Little Diomede	8	**117, 121**	112, **120**	**102, 106**	**204, 208,** 216	**191,** 199, 207
Little Diomede	9	109, 113, **121**	**112,** 116, **120**	**102, 110**	204, **208, 212,** 232	**183,** 191, 199
Little Diomede	10	117, **121,** 125, **133**	**120,** 124	**102**	204, **216, 220,** 232	**183,** 191, 199
Little Diomede	11	**113,** 121, 129, **133**	112, **116, 120**	**102, 106**	**208, 212**,216, 232	**179, 183**
Little Diomede	12	**113, 121,** 129, 133	112, 116, **120**	**102**	**212,** 216, **224,** 228	179, **183,** 187, **211**
Little Diomede	13	**121, 125,** 129	112, **120, 124**	**102, 106**	**204,** 208, **212**	**175,** 179,**183**
Little Diomede	14	117, **121,** 125, **129**	**120,** 124	**102,** 106	**208, 216**	179, **187, 199**
Little Diomede	15	**113, 125**, 129	116, **120**	**102**	**208, 216**, 220, 224	**183, 187,** 191
Little Diomede	16	**121, 125,** 129	112, **120**	**102**	208, **216, 220**	179, 183, **187, 199**
Little Diomede	17	**125,129**	**112, 120**	**102**	208, **212, 216**	**187,** 207, **211**
Little Diomede	18	**121, 133**	104, 112, **120**	**102**	212, **216, 220**	**183,** 187, **207**
Pribilof Islands	1	109, **121, 129**	116, **120**	**102, 114**	208, **216, 220**	179, 183, **199, 211**
Pribilof Islands	2	**125,** 129	112, 116, **120**	**102,** 114	212, **220**	**183, 187**
Pribilof Islands	3	**121, 129**	**112, 116,** 120	**102**	**224,** 228, **232**	**179, 199,** 211
Pribilof Islands	4	**101,** 121, **129**	112, **116, 120**	**102**	**204,** 212,**216,** 220	**183,** 187, **191**
Pribilof Islands	5	**113, 129**	**120, 124**	**102**	**212, 216,** 220, 224	179, **183,** 187
Pribilof Islands	6	**113, 121,** 125	**112,** 120, **124**	98, **102, 110**	**208,** 216, 224, **232**	179, **187,** 191, **215**
Pribilof Islands	7	**113, 121,** 133	**112,** 116, **120**	**102, 106,** 110	208, **220**	179, **187, 191**
Pribilof Islands	8	113, **121, 129,** 133	**112, 116,** 120	**102,** 110	**204,** 208, **212**	**187,** 203, 207
Pribilof Islands	9	**113,** 121, **129,** 133	**112, 120**	**102,** 106	212, **216,** 220	179, **183**
St. Matthews Island	1	**113,** 125, **133**	112, 116, **120**	**102, 110**	**212, 216**	**179,183,** 199
St. Matthews Island	2	109, 113, **125, 129**	112, **116, 120**	**102**	**212,** 216, **220**	179, **183, 211**
St. Matthews Island	3	**109,** 113, **129**	116, **120**	**102, 106**	212, **220,** 228, **236**	179, **183, 207,** 211
St. Matthews Island	4	**105,** 113, **121**	**112,** 116, **120**	**102, 110**	208, **220, 228**	**179**, 219
St. Matthews Island	5	**113,** 121, **129**	**112,** 116, 120	**94, 102,** 106	220, **224, 228**	179, **183,187**
St. Matthews Island	6	109, **125, 129**	**112, 120**	**102**	**216, 220,** 224, 228	**183,** 187,191, **211**
St. Matthews Island	7	**121, 129**	**120**	**102, 110**	**212, 220,** 232	**179, 183,** 199
St. Matthews Island	8	**121, 129**	112, **120**	**102**	**216, 220**	179, **183,**191, **211**
St. Matthews Island	9	109, **129, 133**	**112, 120**	**102**	212, **220,** 224	**159, 183,** 191
St. Matthews Island	10	121, **129**	**112, 120**	**102**	**212, 220,** 224, 232	167, **179, 183,** 199
St. Matthews Island	11	**109, 129**	**112,** 120, **124**	**102**	220, **224, 228**	**167,** 183, 187, **191**
St. Matthews Island	12	**121, 125,** 129	**112, 120**	**102**	**212, 216,** 220, 224	**159, 183**
St. Matthews Island	13	113, **129, 133**	**116, 120**	**102**	212, **216, 220,** 228	**179,** 187, **199,** 207
St. Matthews Island	14	**113, 121,** 125	**112, 116,** 120	**102,** 110	208, **216, 220,** 224	**183**
St. Matthews Island	15	101, **121, 129**	112, 116, **120**	102,**110, 114**	**208,** 216**, 220**	**151,** 183
St. Matthews Island	16	117, **121,** 129	112, 116, **120**	**102,** 110, **134**	212, **216, 220**	179, **183,** 187, **195**
St. Matthews Island	17	**125, 129,** 133	112, **116, 120**	**102, 110**	**208,** 216, 220, **232**	199, **203, 207**

Alleles listed were determined by genotyping both mothers and progeny. Maternal alleles are shown in bold.

Discussion

Diversity and Geographic Population Structure

The strongest geographic pattern in the data is the large genetic divergence and reduced genetic variation of Southeast Alaska BKC relative to other locations. Although there is evidence for moderate genetic divergence among all the collection locations of BKC, Southeast Alaska is consistently the most divergent from the others and carries less genetic variation regardless of the statistical measure used. The distance between Southeast Alaska collections and populations in the Bering Sea may explain the divergence observed between Southeast Alaska collections and other populations. Despite northward currents, BKC larvae may not remain in the water column long enough to be carried and successfully recruit in large numbers into the Bering Sea populations, resulting in the genetic divergence of Southeast Alaska BKC from the other populations.

The low diversity found within Southeast Alaska is consistent with relatively little immigration and smaller population size. There are no BKC abundance or biomass estimates for Southeast Alaska (Stratman et al. 2011), so it is difficult to know how much genetic drift as a result of small population size contributes to the low diversity measures observed in this area. However, ocean currents are unlikely to bring larvae from locations in the Bering Sea or further north and west in the range of BKC into Southeast Alaska (Stabeno et al. 1999, Parada et al. 2010). Rather, currents are dominated by western and northward flows from Southeast Alaska, which would move larvae away

from Southeast Alaska. Whatever the cause, the low BKC genetic diversity measures are consistent with the low diversity measures observed within Southeast Alaska red king crab populations (Vulstek et al. 2013).

The other BKC locations sampled show lower levels of genetic divergence from one another and higher within population genetic variation. All of the Bering Sea collections show low levels of genetic divergence (pairwise *G* - tests, $\hat{F}_{ST}$, and Jost's $\overline{D}$) and cluster closely in the PCA. This is consistent with there being higher levels of gene flow among these populations. The genetic similarity between St. Matthew Island and the Pribilof Islands populations may be due to their close proximity to each other and the northward flow of currents in this portion of the Bering Sea. The Pribilof Islands are approximately 360 km south of St. Matthew, a relatively short distance considering the size of the Bering Sea. Circulation in this area of the Bering Sea is generally northward (Stabeno et al. 1999), meaning transport of larvae from the Pribilof Islands north to St. Matthew Island is possible. The combination of a relatively short distance between the two populations, the long larval duration time exhibited by BKC, and favorable currents for moving Pribilof Island larvae northward in the Bering Sea could explain why these populations are more similar to each other than they are to other populations within the study area. The northward movement of Bering Sea surface water may also explain why the Chaunskaya Bay population clusters closely with the St. Matthew and Pribilof Island populations in the PCA. This northward flow may move larval BKC from the Island populations through the Bering Strait, providing gene flow into northern populations.

Temporal Population Structure

The reasons behind the observed changes in allele frequencies over time at both the Pribilof Islands and St Matthews Island are unclear. These temporal changes, taking place within two generations, suggest genetic drift plays an important role in these populations. This genetic drift could be a consequence of the large drops in biomass seen over time leading to small numbers of breeders. However, we cannot eliminate the possibility that variation over time in where BKC were collected for the St. Matthew Island and the Pribiliof Islands samples contributed to the temporal divergence observed.

A recent study of California spiny lobster reported low genetic population structure when using traditional F_{ST} measures, but much higher levels of structuring in some areas when kinship analysis was used (Iacchei et al. 2013). Similar to the temporal pairwise comparison test of BKC, Iacchei et al. (2013) reported low pairwise F_{ST} estimates in American lobster. A significantly higher than expected proportion of kin were found within the majority of study sites, suggesting much higher genetic structuring in some areas than suggested by their F_{ST} measure estimates. The increased genetic population structure in specific areas was reported to being due to high retention of larvae from a small number of broods (Iacchei et al. 2013). If large numbers of BKC siblings settle at specific sites, and only larvae from a small number of broods are retained at these sites, then the slight, but significant, changes in temporal pairwise homogeneity tests found at the Pribilof Islands and St. Matthew Island may be the result of sweepstakes recruitment. Sweepstakes recruitment occurs when a relatively small number of females in a

population contribute offspring to the future pool of reproductive adults (Hedgecock 1994).

If the temporal changes observed at the Pribilof Islands and St. Matthew Island are the result of genetic drift, overharvesting of BKC in the 1980s and early 1990s could have contributed to this pattern. Overharvesting causes a decrease in population size, and genetic drift acts more strongly on small populations. With few adults available to spawn in small populations, the likelihood of broods from any individual female "winning" these sweepstakes recruitment events increases. This would cause individuals at a given site to be closely related to each other and could cause allele frequencies to change over time (Iacchei et al. 2013).

Bottlenecks could also account for rapid changes in allele frequencies, though no evidence of a bottleneck was found at either temporally sampled location. Despite a lack of evidence for population bottlenecks, the changes in allele frequencies over time are consistent both with genetic drift as a result of reductions in adult breeding biomass and with recruitment from distant populations. Distinguishing the relative influence of these two scenarios on allele frequencies will require combined ecological and genetic studies (Waples 1998). However, it is clear that there has not been sufficient gene flow from other populations to rebuild the Pribilof Islands and St. Matthew Island populations to previous abundances.

Temporal vs. Geographic Population Structure

Although temporal variation in allele frequencies was observed in the Pribilof Islands and St. Matthews Island collections, variation in allele frequencies among geographic locations was generally higher. Temporal pairwise $\hat{F}_{ST}$ values ranged from 0.0038 to 0.0255, whereas geographic pairwise $\hat{F}_{ST}$ values ranged from 0.0045 to 0.0954. This pattern was supported by the PCA results as well, which showed that geographic distances dominated differences in allele frequencies. Therefore, even though the Pribilof Islands and St. Matthews Island collections exhibit changes in allele frequencies over short time frames, the broad geographic patterns of differentiation among populations across the study area are consistent.

Mating Structure

The single paternity mating structure observed in BKC is consistent with the lack of sperm storage compartments in females and has implications for broodstock selection. Female BKC mating with a single male means a larger number of broodstock would be needed to successfully mimic the N_e of the wild population should enhancement ever occur. If broodstock N_e is too low, loss of genetic variation may occur as well as negative effects from inbreeding that are present in small populations (Schultz & Lynch 1997). Low genetic variation and increased inbreeding of hatchery – released crab could have negative impacts on the enhanced population. If the goal of the enhancement program was to increase the wild population to a level where it could be self-sustaining and support a future fishery, lowering genetic variation and increasing inbreeding might

counteract this goal. It is therefore very important that a sufficiently large number of broodstock be used should enhancement occur.

Comparison to Other Crab Species

Levels of differentiation among BKC populations are similar to those found in red king crab. Collection sites for BKC were similar to collection sites for red king crab and overall $\hat{F}_{ST}$ = 0.025 (SE = 0.009) in red king crab was similar to the overall $\hat{F}_{ST}$ = 0.027 (SE = 0.005) found in BKC. Overall estimates of $\overline{D}$ ($\overline{D}$ = 0.074 for red king crab and $\overline{D}$= 0.091 in BKC) and $\overline{G'}_{ST}$ ($\bar{G}_{ST}$ = 0.128 for red king crab and $\overline{G'}_{ST}$ = 0.120 for BKC) are also similar (Vulstek et al. 2013), suggesting that these consistent genetic patterns reflect underlying similarities in life history rather than being artifacts of any specific measure of population divergence. In addition, red king crab show the same general pattern as BKC of relatively low levels of genetic diversity in Southeast Alaska and high divergence between Southeast Alaska and other Alaskan populations to the north and west. Red king crab also exhibit a mating system of single paternity of each brood (Vulstek 2013).

Other crab that occur within the population range of BKC and share similar larval duration times generally exhibited lower levels of differentiation than did BKC. Microsatellite analysis of snow crab in Alaska revealed $\overline{D}$ = 0.004 (Mincks Hardy et al. 2011), which is more than an order of magnitude lower than that observed in BKC in this study. Allozyme data from Tanner crab in Alaska provided a $\hat{F}_{ST}$value estimate of 0.0046 (Merkouris et al. 1998).

A 2008 study using microsatellite markers reported Dungeness crab in British Columbia had similar $\hat{F}_{ST}$ ($\hat{F}_{ST}$ = 0.031) to BKC ($\hat{F}_{ST}$ = 0.027; Beacham et al. 2008).

General Conclusions

This study suggests there is gene flow among BKC populations in the eastern Bering Sea and throughout the area sampled. However, significant genetic divergence was found among all of the geographic areas sampled, and the pattern of increased genetic distance with physical distance is consistent across the study area. Clearly, Southeast Alaska BKC show the greatest divergence from other populations in Alaska and Russia, and the other BKC populations diverge from one another to a lesser extent. Results of this study suggest that BKC genetic population structure occurs at the level at which samples were collected. Alaskan populations of BKC should therefore be managed at this geographic scale.

This study does not provide a definitive answer as to why the Pribilof Islands population continues to decline while the St. Matthew Island population has been declared recovered. The changes in allele frequencies observed over time in these two locations are consistent with non-exclusive impacts of large effects of genetic drift as a result of low breeding adult biomass and gene flow from other populations.

These temporal genetic changes should continue to be studied in order to determine the cause of these temporal changes, as well as to inform managers of necessary changes to broodstock composition should enhancement occur. If enhancement is used as a management tool, the single paternity mating system of BKC should be incorporated into determinations of the number of individuals needed to create enhancement broodstock,

and the pattern of increased genetic distance with geographic distance should be taken into considerations of where broodstock are collected.

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Appendix A

Supporting data for genetics studies

Table A-1: Sample coordinates (when available) of blue king crab collection locations.

Latitude	Longitude	Geographic Location
65.44503	W 168.5635	Little Diomede, Alaska USA
56.67039	W 169.50622	Pribilof Islands, Alaska USA
56.9965	W 169.55851	Pribilof Islands, Alaska USA
57.00965	W 168.94564	Pribilof Islands, Alaska USA
57.01172	W 170.01364	Pribilof Islands, Alaska USA
57.08369	W 170.12933	Pribilof Islands, Alaska USA
57.1733	W 169.30993	Pribilof Islands, Alaska USA
57.32261	W 168.9785	Pribilof Islands, Alaska USA
57.33163	W 168.98487	Pribilof Islands, Alaska USA
57.50592	W 169.3521	Pribilof Islands, Alaska USA
59.00425	W 174.41502	St. Matthew Island, Alaska USA
59.00897	W 175.02485	St. Matthew Island, Alaska USA
59.00925	W 173.0988	St. Matthew Island, Alaska USA
59.33459	W 175.09661	St. Matthew Island, Alaska USA
59.6613	W 173.27832	St. Matthew Island, Alaska USA
59.83018	W 172.3004	St. Matthew Island, Alaska USA
59.83052	W 173.556	St. Matthew Island, Alaska USA
59.83323	W 174.20117	St. Matthew Island, Alaska USA
59.84002	W 172.89925	St. Matthew Island, Alaska USA
59.98373	W 172.55498	St. Matthew Island, Alaska USA
59.98632	W 174.5768	St. Matthew Island, Alaska USA
59.99324	W 172.608	St. Matthew Island, Alaska USA
60.01232	W 173.96047	St. Matthew Island, Alaska USA
60.01503	W 171.9769	St. Matthew Island, Alaska USA
60.11752	W 173.7704	St. Matthew Island, Alaska USA
60.15322	W 172.97217	St. Matthew Island, Alaska USA
60.15398	W 172.30388	St. Matthew Island, Alaska USA
60.18597	W 174.34717	St. Matthew Island, Alaska USA
60.1879	W 173.0388	St. Matthew Island, Alaska USA
60.28822	W 173.38235	St. Matthew Island, Alaska USA
60.33813	W 174.05553	St. Matthew Island, Alaska USA
60.35162	W 172.06122	St. Matthew Island, Alaska USA
69.83333	E 170	Chaunskaya Bay, Russia
56.11667	E 154.28333	South Shelikhov Gulf, Russia (1998)
58.73333	E 156.28333	North Shelikhov Gulf, Russia (1999)
60.58 -60.98	E 171.55 - 171.73	Western Bering Sea, Siberia Russia

Table A-2: Loadings from the first two principal components from PCA of blue king crab allele frequency data.

Locus	Allele	PC1	PC2
L8	146	0.0080673027	0.000646
L8	154	0.004148	-0.016493
L8	158	0.001852	0.000646
L8	162	0.005332	-0.003343
L8	166	0.010132	-0.003417
L8	170	0.006177	0.004965
L8	174	0.003979	-0.022407
L8	178	0.003598	0.009572
L8	182	-0.001648	0.029416
L8	186	-0.042713	0.144557
L8	190	-0.233881	-0.116746
L8	194	0.070573	0.068350
L8	198	0.009500	-0.015520
L8	202	0.053581	-0.042711
L8	206	0.070062	-0.042954
L8	210	0.021656	-0.001729
L8	214	0.021372	-0.003724
L8	218	-0.004056	0.008539
L8	222	-0.001518	0.002351
L19	94	0.003851	0.013283
L19	98	-0.001274	0.001903
L19	102	-0.205699	-0.102753
L19	106	-0.021649	0.023554
L19	110	0.238304	0.012139
L19	114	0.018821	0.069580
L19	118	0.012286	-0.015503
L19	126	-0.000791	0.001226
L19	130	0.003610	-0.001769
L19	134	-0.044722	-0.005489
L19	138	-0.002736	0.003829
L21	101	-0.023587	0.017872

Locus	Allele	PC1	PC2
L21	105	-0.001449	0.004639
L21	109	-0.048186	-0.009885
L21	113	0.058521	-0.138655
L21	117	0.052790	0.098729
L21	121	-0.098657	-0.087236
L21	125	0.063366	0.028588
L21	129	0.042284	0.115465
L21	133	-0.068575	-0.033620
L21	137	0.025185	0.000445
L21	141	-0.001692	0.003661
L31	147	-0.002478	0.003839
L31	151	-0.003631	0.006207
L31	159	0.018502	-0.010873
L31	163	-0.001395	0.002489
L31	167	0.002547	0.020736
L31	171	0.008985	0.021267
L31	175	-0.002635	-0.002313
L31	179	0.335429	0.179694
L31	183	-0.005682	-0.144560
L31	187	-0.046578	0.099192
L31	191	0.058430	-0.036775
L31	195	-0.024237	0.000254
L31	199	-0.122291	-0.008079
L31	203	-0.027857	0.019562
L31	207	-0.095490	-0.086732
L31	211	-0.084902	-0.081907
L31	215	0.000221	-0.006002
L31	219	-0.002415	0.014836
L31	223	-0.004263	0.008031
L31	227	-0.000260	0.001135
L39	200	-0.001488	0.013066
L39	204	-0.050062	-0.005559
L39	208	0.016294	0.038000
L39	212	0.108687	0.025418
L39	216	0.080640	-0.318838
L39	220	-0.120987	0.009799
L39	224	-0.026740	0.179792
L39	228	-0.041053	-0.018932

Locus	Allele	PC1	PC2
L39	232	0.037112	0.056659
L39	236	-0.003504	0.012404
L39	248	0.001102	0.008189
L40	108	0.014553	-0.001643
L40	112	0.288408	-0.262390
L40	116	0.054252	-0.027607
L40	120	-0.372003	0.395146
L40	124	0.014925	-0.104094
L40	132	-0.000135	0.000587
L18	115	0.003608	-0.023319
L18	119	0.028936	0.004555
L18	123	0.091398	0.007650
L18	127	-0.195333	0.102062
L18	131	-0.048987	-0.121211
L18	135	0.007342	-0.003603
L18	139	0.095558	-0.217806
L18	143	0.061593	0.218761
L18	147	-0.033992	-0.001182
L18	151	-0.009283	0.032789
L18	155	-0.000841	0.001303
L32	111	-0.000146	0.000636
L32	115	0.011450	0.020445
L32	119	0.062110	-0.017205
L32	123	-0.009480	-0.008704
L32	127	0.154329	0.070713
L32	131	0.066464	-0.118964
L32	135	-0.049624	-0.061778
L32	139	0.068893	-0.097052
L32	143	0.004872	0.093974
L32	147	-0.002147	-0.002117
L32	151	-0.056330	-0.004670
L32	155	0.016990	0.049803
L32	159	0.098023	0.315016
L32	163	-0.259832	-0.215990
L32	167	-0.059338	-0.053284
L32	171	-0.019745	0.051110
L32	175	-0.027974	-0.023823
L32	179	0.001631	0.001255

Locus	Allele	PC1	PC2
L32	195	-0.000146	0.000636
L9	82	0.009943	-0.007317
L9	86	0.017498	-0.053879
L9	90	0.139236	-0.081117
L9	94	0.036057	0.061876
L9	98	0.183194	0.194031
L9	102	-0.346783	0.010627
L9	106	-0.066358	-0.072252
L9	110	-0.006597	-0.016003
L9	114	-0.003165	-0.002602
L9	118	-0.001142	0.001329
L9	122	0.000544	-0.001983
L9	126	0.017360	-0.015122
L9	130	0.010682	-0.009445
L9	134	0.009532	-0.008142
L27	72	0.005405	-0.003723
L27	76	0.008987	-0.005457
L27	88	0.000689	0.001999
L27	92	0.020248	0.030054
L27	96	-0.094310	-0.180268
L27	104	0.058377	0.140827
L27	108	0.008476	0.009310
L27	112	0.001635	0.001044
L27	116	-0.008711	0.004980
L27	140	-0.000798	0.001236

Loadings obtained from acrsine square root transformed allele frequencies.

Table A-3: Descriptive statistics of blue king crab microsatellite data at ten loci.

		Locus									
Population	Value	L8	L19	L21	L31	L39	L40	L18	L32	L9	L27
9396PI	*n*	109	110	109	111	110	110	110	110	110	108
	n_a	10	8	11	13	8	4	8	15	11	8
	min_a	170	102	109	163	208	112	127	127	86	96
	max_a	210	134	141	219	236	124	151	175	134	116
93SMI	*n*	60	59	59	60	60	57	60	60	60	60
	n_a	11	6	10	14	9	5	9	12	9	5
	min_a	166	102	109	179	208	112	127	127	94	96
	max_a	214	138	137	223	236	124	151	171	130	112
SSG	*n*	36	38	35	38	38	38	38	38	38	38
	n_a	14	6	7	7	6	4	9	9	9	5
	min_a	154	102	105	159	204	108	115	119	86	92
	max_a	214	134	133	215	232	124	147	167	134	116
NSG	*n*	39	39	33	39	39	39	39	39	39	39
	n_a	10	7	7	6	7	5	7	10	11	5
	min_a	166	94	113	159	204	108	119	115	82	72
	max_a	214	130	137	191	232	124	147	167	134	108
CSB	*n*	47	49	50	48	50	49	50	50	50	50
	n_a	13	6	9	9	8	4	10	13	6	4
	min_a	166	94	101	167	204	112	115	115	86	92
	max_a	218	134	137	211	248	124	151	175	106	108
LD	*n*	18	18	18	18	18	18	18	18	18	18
	n_a	12	5	7	8	8	4	8	10	7	4
	min_a	154	102	109	175	204	112	115	123	86	92
	max_a	218	118	133	211	232	124	147	167	122	116
PI	*n*	112	112	112	112	112	112	112	112	112	112
	n_a	15	10	10	15	10	5	10	16	7	8
	min_a	154	94	101	147	200	108	119	115	86	76
	max_a	222	138	137	223	236	124	155	175	110	140
SEAK	*n*	38	36	37	39	38	38	38	38	38	37
	n_a	7	4	7	8	7	3	8	8	6	2
	min_a	174	102	101	183	204	112	115	131	86	96
	max_a	218	134	133	215	232	124	147	175	110	104
SMI	*n*	239	242	239	242	207	236	222	221	220	236
	n_a	15	7	11	18	10	6	10	18	9	8
	min_a	154	94	101	151	200	108	115	111	86	72
	max_a	214	134	141	227	236	132	151	195	122	116
WBS	*n*	47	47	46	49	48	49	49	49	49	49
	n_a	13	7	7	12	9	5	7	11	7	4
	min_a	146	94	101	167	200	108	115	119	86	88
	max_a	214	134	133	219	236	124	143	179	122	108

Data includes sample size (*n*), number of alleles (n_a),minimum allele size (min_a) and maximum allele size (max_a) at each locus. Populations: 9396_PI (Pribilof Island samples collected in 1993 and 1996), 93_SMI (St. Matthew Island samples collected in 1993), SSG (South Shelikhov Gulf), NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay) LD (Little Diomede), PI (Pribilof Islands), SEAK (Southeast Alaska), SMI (St. Matthew Island), and WBS (Western Bering Sea).

Table A-4: Ten blue king crab specific microsatellite primers, sequences and thermal profiles used in the study.

Locus	Primer	Sequence	Thermal Profile
L8	F: CCACGAAGTCCTTGACCACG R: CAGAGCCCTGAAACCATTACTAGC	ATAC(56)	95°C (5 min); 40 cycles of [94°C (30s) + 67°C (30 sec) + 72°C (30s)]; 72°C (10min)
L19	F: GCGATTACGCTGGAGGTAGG R: CACACTGTTCACCTATAATATCCCG	ATAC(36) ATAC(36)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) +55°C (30 sec) +72°C (30s)]; 72°C (10min)
L21	F: GGCCAGTGATTCATAAACCG R: TCTGGTGGGTTTCATTGAGC	TCTG(44)	95°C (5 min); 40 cycles of [94°C (30s) + 65°C (30 sec) + 72°C (30s)]; 72°C (10min)
L31	F: TCGCCTAGGCAGGATATGG R: TTTCCCATTTAAATTCCAAAGC	ATAC(52)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L39	F: TGAATTACACGCAGTATTTATATCCACC R: TGAGTTAAATAAATTCTGGACAACAAGG	ATCT(52)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)] + 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L40	F: TGCTAATGAAGGAGGCCTGG R: CACAAGTCCTACACACTTCATTTCC	AATG(36) AATG(36)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)] + 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L18	F: GCCACATCACGTAAATGAATAGG R:GCTGCTCTCTCCTTGTTGGG	ATCT(40) ATCT(48)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L32	F: TCTATCCTTCCAGGAATCTGCC R: CTGTCGTCATACCTGGCTGC	ATAC(52)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L9	F: CCCTTATTAACGCTTCCATCCC R: CAAAGTACAACTGTTAGGAGAGAGGG	AGTG(28) AGTG(40)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) + 55°C (30 sec) + 72°C (30s)]; 72°C (10min)
L27	F: GGGCTTCGATTATCGGTTCG R: AAGAGTCGCGTGTCAGAGGG	ATCT(48) ATCT(32)	95°C (5 min); 20 cycles of [95°C (30s) + 65°C (30 sec) + 72°C (30s)]+ 20 cycles of [95°C (30s) +55°C (30 sec) + 72°C (30s)]; 72°C (10min)

Table A-5: Hardy-Weinberg Expectations for ten blue king crab collection locations at ten loci.

Population	Locus	*P*- value	Population	Locus	*P*- value
1993 SMI	L8	0.1735	CSB	L8	0.0783
	L19	0.9325		L19	0.5068
	L21	0.2215		L21	0.3743
	L31	0.7607		L31	0.2616
	L39	0.5752		L39	0.3178
	L40	0.6022		L40	0.38
	L18	0.7971		L18	0.0134
	L32	0.0071		L32	0.0889
	L9	0.934		L9	0.4435
	L27	0.9258		L27	0.1545
9396 PI	L8	0.0419	LD	L8	0.0797
	L19	0.5097		L19	1.0000
	L21	0.0456		L21	0.9795
	L31	0.0708		L31	0.1332
	L39	0.9106		L39	0.918
	L40	0.5618		L40	0.6173
	L18	0.316		L18	0.6869
	L32	0.1093		L32	0.4141
	L9	0.0971		L9	0.0296
	L27	0.9916		L27	0.1594

Population	**Locus**	***P*- value**	**Population**	**Locus**	***P*- value**
SSG	L8	0.9099	PI	L8	0.2002
	L19	0.4298		L19	0.0749
	L21	0.2068		L21	0.0101
	L31	0.9200		L31	< 0.001*
	L39	0.1658		L39	0.0073
	L40	0.2617		L40	0.5931
	L18	0.0492		L18	0.9692
	L32	0.7878		L32	0.9209
	L9	0.3696		L9	0.0882
	L27	0.2798		L27	0.4994
NSG	L8	0.4062	SEAK	L8	0.8020
	L19	0.7585		L19	0.2868
	L21	0.4738		L21	0.0032*
	L31	0.6943		L31	0.0555
	L39	0.3961		L39	0.5555
	L40	0.8274		L40	0.3878
	L18	0.5349		L18	0.4325
	L32	0.0431		L32	0.8520
	L9	0.078		L9	0.1055
	L27	0.0178		L27	0.3218

Population	**Locus**	***P*- value**
SMI	L8	0.0007*
	L19	0.1030
	L21	0.1249
	L31	0.4256
	L39	0.2416
	L40	0.8193
	L18	0.4359
	L32	0.3658
	L9	< 0.001*
	L27	0.0119
WBS	L8	0.0450
	L19	0.9500
	L21	0.6861
	L31	0.0910
	L39	0.9853
	L40	0.0057
	L18	0.0254
	L32	0.0328
	L9	0.6047
	L27	0.1787

*Indicates the stated value is still significant after being corrected for multiple tests with a sequential Bonferrnoi correction. Populations: 9396_PI (Pribilof Island samples collected in 1993 and 1996), 93_SMI (St. Matthew Island samples collected in 1993), SSG (South Shelikhov Gulf), NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay) LD (Little Diomede), PI (Pribilof Islands), SEAK (Southeast Alaska), SMI (St. Matthew Island), and WBS (Western Bering Sea).

Table A-6: Hierarchical test for blue king crab samples at various spatial scales.

Location	*G*	df	P_G	*F*	P_F
93_PI	-	-	-		
96_PI	-	-	-		
Within	-	-	-		
Between	68.1895	44	1.1160E-02		
				1.0000	5.0000E-01
HPI_HSMI	196.4925	96	6.8207E-09		
PI_SMI	157.6835	51	7.9196E-13		
Within	354.1760	147	3.9209E-19		
Between	138763.0614	51	0.0000E+00		
				1129.2808	1.5959E-168*
PI	100.4961	30	1.5501E-09		
SMI	83.0259	29	4.1423E-07		
Within	183.5220	59	1.0704E-14		
Between	71.4774	29	1.8987E-05		
				0.7924	7.5003E-01
SEAK	-	-	-		
EBS	143.1244	58	3.7806E-09		
Within	143.1244	58	3.7806E-09		
Between	283.3154	29	1.5859E-43		
				3.9590	4.1112E-06*
Alaska	574.3130	145	2.0470E-52		
Russia	252.4310	87	4.9570E-18		
Within	826.7439	232	1.0689E-67		
Between	391.0854	29	4.7432E-65		
				3.7843	6.3751E-09*

The *G* column reports the *G* – test estimates of variation within a specific location, variation within both locations (Within), and variation between the two locations (Between). The P_G column reports the *P* – value for the *G* – test. The *F* column reports estimates of *F* – tests between the Within and Between estimates. The P_G column indicates the *P* – value for the associated *F* – test. * indicates a significant *P* – value for the associated F – test. Locations: Historic (1990s) Pribilof Islands and St. Matthew Island samples combined (HPI_HSMI), Pribilof Islands (PI), St. Matthew Island (SMI), Southeast Alaska (SEAK), all sampled locations within the eastern Bering Sea combined (EBS), all sampled Alaskan locations combined (Alaska), all sampled Russian locations combined (Russia).

Table A-7: *P*-values across all blue king crab populations for Fisher's Exact Test.

Locus	*P*-value
L8	0.96304
L19	0.19142
L21	0.96724
L31	0.91994
L39	0.01582*
L40	0.05681
L18	0.20910
L32	0.28118
L9	0.29742
L27	0.49391

*Indicates a significant *P* – value for the associated test.

Table A-8: Blue king crab sample sizes by location and molt year for Fisher's exact test.

Population	Molt Year	*n*
Pribilof Islands	Even	9
Pribilof Islands	Odd	18
St. Matthew Island	Even	11
St. Matthew Island	Odd	49

Table A-9: Linkage Disequilibrium *P*-values by population and pair of loci.

Population	Locus # 1	Locus # 2	*P* -Value	Population	Locus # 1	Locus # 2	*P* -Value
93SMI	L8	L19	0.684460	93SMI	L39	L27	0.316850
93SMI	L8	L21	0.949619	93SMI	L40	L27	0.943300
93SMI	L19	L21	0.321141	93SMI	L18	L27	0.631184
93SMI	L8	L31	0.785366	93SMI	L32	L27	0.883536
93SMI	L19	L31	0.215733	93SMI	L9	L27	0.403277
93SMI	L21	L31	0.948190	9396PI	L8	L19	0.089259
93SMI	L8	L39	0.816744	9396PI	L8	L21	0.239679
93SMI	L19	L39	0.050456	9396PI	L19	L21	0.113373
93SMI	L21	L39	0.216012	9396PI	L8	L31	0.930195
93SMI	L31	L39	0.089016	9396PI	L19	L31	0.913926
93SMI	L8	L40	0.795982	9396PI	L21	L31	0.682036
93SMI	L19	L40	0.942221	9396PI	L8	L39	0.165580
93SMI	L21	L40	0.108284	9396PI	L19	L39	0.656960
93SMI	L31	L40	0.449378	9396PI	L21	L39	0.036057
93SMI	L39	L40	0.904318	9396PI	L31	L39	0.960783
93SMI	L8	L18	0.785348	9396PI	L8	L40	0.085908
93SMI	L19	L18	0.899955	9396PI	L19	L40	0.095162
93SMI	L21	L18	0.519132	9396PI	L21	L40	0.815305
93SMI	L31	L18	0.206577	9396PI	L31	L40	0.298210
93SMI	L39	L18	0.140697	9396PI	L39	L40	0.763739
93SMI	L40	L18	0.488210	9396PI	L8	L18	0.031673
93SMI	L8	L32	0.610154	9396PI	L19	L18	0.220018
93SMI	L19	L32	0.864873	9396PI	L21	L18	0.027664
93SMI	L21	L32	0.560891	9396PI	L31	L18	0.298801
93SMI	L31	L32	0.110129	9396PI	L39	L18	0.299041
93SMI	L39	L32	0.931328	9396PI	L40	L18	0.212797
93SMI	L40	L32	0.044638	9396PI	L8	L32	0.829563
93SMI	L18	L32	0.566682	9396PI	L19	L32	0.587838
93SMI	L8	L9	0.797351	9396PI	L21	L32	0.243558
93SMI	L19	L9	0.024301	9396PI	L31	L32	0.422380
93SMI	L21	L9	0.138743	9396PI	L39	L32	0.299418
93SMI	L31	L9	0.820751	9396PI	L40	L32	0.115706
93SMI	L39	L9	0.828287	9396PI	L18	L32	0.748209
93SMI	L40	L9	0.079147	9396PI	L8	L9	0.991376
93SMI	L18	L9	0.967402	9396PI	L19	L9	0.477424
93SMI	L32	L9	0.360601	9396PI	L21	L9	0.769925
93SMI	L8	L27	0.434302	9396PI	L31	L9	0.612691
93SMI	L19	L27	0.048114	9396PI	L39	L9	0.454691
93SMI	L21	L27	0.230466	9396PI	L40	L9	0.909201
93SMI	L31	L27	0.829351	9396PI	L18	L9	0.943264

Population	Locus # 1	Locus # 2	*P*-Value	Population	Locus # 1	Locus # 2	*P*-Value
9396PI	L32	L9	0.281617	SSG	L19	L9	0.432890
9396PI	L8	L27	0.622305	SSG	L21	L9	0.618960
9396PI	L19	L27	0.417394	SSG	L31	L9	0.285389
9396PI	L21	L27	0.090055	SSG	L39	L9	0.479281
9396PI	L31	L27	0.969657	SSG	L40	L9	0.404429
9396PI	L39	L27	0.944603	SSG	L18	L9	0.567439
9396PI	L40	L27	0.789388	SSG	L32	L9	0.584632
9396PI	L18	L27	0.046020	SSG	L8	L27	0.603446
9396PI	L32	L27	0.478425	SSG	L19	L27	0.849395
9396PI	L9	L27	0.423845	SSG	L21	L27	0.527551
SSG	L8	L19	0.811316	SSG	L31	L27	0.610958
SSG	L8	L21	0.203343	SSG	L39	L27	0.035773
SSG	L19	L21	0.044940	SSG	L40	L27	0.063132
SSG	L8	L31	0.941364	SSG	L18	L27	0.226094
SSG	L19	L31	0.156136	SSG	L32	L27	0.901882
SSG	L21	L31	0.032636	SSG	L9	L27	0.883221
SSG	L8	L39	0.072639	NSG	L8	L19	0.054489
SSG	L19	L39	0.086731	NSG	L8	L21	0.034689
SSG	L21	L39	0.545361	NSG	L19	L21	0.138464
SSG	L31	L39	0.014713	NSG	L8	L31	0.624520
SSG	L8	L40	0.880476	NSG	L19	L31	0.743131
SSG	L19	L40	0.242973	NSG	L21	L31	0.963989
SSG	L21	L40	0.063332	NSG	L8	L39	0.415167
SSG	L31	L40	0.531654	NSG	L19	L39	0.608795
SSG	L39	L40	0.617426	NSG	L21	L39	0.033222
SSG	L8	L18	0.570676	NSG	L31	L39	0.019945
SSG	L19	L18	0.049563	NSG	L8	L40	0.116455
SSG	L21	L18	0.817974	NSG	L19	L40	0.046195
SSG	L31	L18	0.302980	NSG	L21	L40	0.047296
SSG	L39	L18	0.796397	NSG	L31	L40	0.537479
SSG	L40	L18	0.887944	NSG	L39	L40	0.311126
SSG	L8	L32	0.100335	NSG	L8	L18	0.004462
SSG	L19	L32	0.287844	NSG	L19	L18	0.330296
SSG	L21	L32	1.000000	NSG	L21	L18	0.213739
SSG	L31	L32	0.425750	NSG	L31	L18	0.164959
SSG	L39	L32	0.187401	NSG	L39	L18	0.195900
SSG	L40	L32	0.525992	NSG	L40	L18	0.749443
SSG	L18	L32	0.159621	NSG	L8	L32	0.943754
SSG	L8	L9	0.263810	NSG	L19	L32	0.992561

Population	Locus # 1	Locus # 2	*P*-Value	Population	Locus # 1	Locus # 2	*P*-Value
NSG	L21	L32	0.345652	CSB	L21	L18	0.873948
NSG	L31	L32	0.900473	CSB	L31	L18	0.137481
NSG	L39	L32	0.840788	CSB	L39	L18	0.233311
NSG	L40	L32	0.029399	CSB	L40	L18	0.988312
NSG	L18	L32	0.611883	CSB	L8	L32	0.579195
NSG	L8	L9	0.401768	CSB	L19	L32	0.205697
NSG	L19	L9	0.955907	CSB	L21	L32	0.918937
NSG	L21	L9	0.326978	CSB	L31	L32	0.352824
NSG	L31	L9	0.581565	CSB	L39	L32	0.167726
NSG	L39	L9	0.930868	CSB	L40	L32	0.438751
NSG	L40	L9	0.447537	CSB	L18	L32	0.217899
NSG	L18	L9	0.958469	CSB	L8	L9	0.263115
NSG	L32	L9	0.646813	CSB	L19	L9	0.056850
NSG	L8	L27	0.544487	CSB	L21	L9	0.486186
NSG	L19	L27	0.503984	CSB	L31	L9	0.871021
NSG	L21	L27	0.719840	CSB	L39	L9	0.374769
NSG	L31	L27	0.800217	CSB	L40	L9	0.097878
NSG	L39	L27	0.493476	CSB	L18	L9	0.817989
NSG	L40	L27	0.948299	CSB	L32	L9	0.935592
NSG	L18	L27	0.490696	CSB	L8	L27	0.841325
NSG	L32	L27	0.742876	CSB	L19	L27	0.412886
NSG	L9	L27	0.432997	CSB	L21	L27	0.110072
CSB	L8	L19	0.351120	CSB	L31	L27	0.896844
CSB	L8	L21	0.843452	CSB	L39	L27	0.255251
CSB	L19	L21	0.876696	CSB	L40	L27	0.239329
CSB	L8	L31	0.318001	CSB	L18	L27	0.446129
CSB	L19	L31	0.524005	CSB	L32	L27	0.522549
CSB	L21	L31	0.935957	CSB	L9	L27	0.102545
CSB	L8	L39	0.134081	LD	L8	L19	0.193552
CSB	L19	L39	0.920292	LD	L8	L21	1.000000
CSB	L21	L39	0.993522	LD	L19	L21	0.747756
CSB	L31	L39	0.365929	LD	L8	L31	0.205649
CSB	L8	L40	0.129462	LD	L19	L31	0.871482
CSB	L19	L40	0.826868	LD	L21	L31	1.000000
CSB	L21	L40	0.060528	LD	L8	L39	0.325454
CSB	L31	L40	0.093079	LD	L19	L39	1.000000
CSB	L39	L40	0.503529	LD	L21	L39	1.000000
CSB	L8	L18	0.864105	LD	L31	L39	1.000000
CSB	L19	L18	0.279771	LD	L8	L40	0.817110

Population	Locus # 1	Locus # 2	*P*-Value	Population	Locus # 1	Locus # 2	*P*-Value
LD	L19	L40	0.883079	PI	L21	L31	0.722875
LD	L21	L40	0.803444	PI	L8	L39	0.418520
LD	L31	L40	1.000000	PI	L19	L39	0.689310
LD	L39	L40	0.125294	PI	L21	L39	0.532551
LD	L8	L18	0.873317	PI	L31	L39	0.682649
LD	L19	L18	0.040786	PI	L8	L40	0.866654
LD	L21	L18	1.000000	PI	L19	L40	0.211697
LD	L31	L18	1.000000	PI	L21	L40	0.102126
LD	L39	L18	1.000000	PI	L31	L40	0.195997
LD	L40	L18	0.616499	PI	L39	L40	0.512051
LD	L8	L32	0.030328	PI	L8	L18	0.601866
LD	L19	L32	1.000000	PI	L19	L18	0.700967
LD	L21	L32	1.000000	PI	L21	L18	0.510527
LD	L31	L32	0.170273	PI	L31	L18	0.151975
LD	L39	L32	1.000000	PI	L39	L18	0.927110
LD	L40	L32	0.529011	PI	L40	L18	0.331062
LD	L18	L32	1.000000	PI	L8	L32	0.340353
LD	L8	L9	0.383387	PI	L19	L32	0.066242
LD	L19	L9	1.000000	PI	L21	L32	0.464915
LD	L21	L9	0.430202	PI	L31	L32	0.827024
LD	L31	L9	0.062217	PI	L39	L32	0.195755
LD	L39	L9	0.605636	PI	L40	L32	0.133486
LD	L40	L9	0.921044	PI	L18	L32	0.127957
LD	L18	L9	0.993998	PI	L8	L9	0.033792
LD	L32	L9	0.609538	PI	L19	L9	0.139049
LD	L8	L27	0.915876	PI	L21	L9	0.172247
LD	L19	L27	0.605455	PI	L31	L9	0.923190
LD	L21	L27	0.627101	PI	L39	L9	0.123456
LD	L31	L27	0.814120	PI	L40	L9	0.883177
LD	L39	L27	1.000000	PI	L18	L9	0.904441
LD	L40	L27	0.104510	PI	L32	L9	0.862416
LD	L18	L27	0.531597	PI	L8	L27	0.279315
LD	L32	L27	1.000000	PI	L19	L27	0.456183
LD	L9	L27	0.972247	PI	L21	L27	0.990758
PI	L8	L19	0.630699	PI	L31	L27	0.278443
PI	L8	L21	0.081542	PI	L39	L27	0.434413
PI	L19	L21	0.972831	PI	L40	L27	0.735456
PI	L8	L31	0.544654	PI	L18	L27	0.975346
PI	L19	L31	0.823305	PI	L32	L27	0.233561

Population	Locus # 1	Locus # 2	*P*-Value	Population	Locus # 1	Locus # 2	*P*-Value
PI	L9	L27	0.885532	SEAK	L31	L27	0.676731
SEAK	L8	L19	0.322168	SEAK	L39	L27	0.522613
SEAK	L8	L21	0.764063	SEAK	L40	L27	0.709746
SEAK	L19	L21	0.836388	SEAK	L18	L27	0.418751
SEAK	L8	L31	0.547029	SEAK	L32	L27	0.004101
SEAK	L19	L31	0.125857	SEAK	L9	L27	0.057993
SEAK	L21	L31	0.388825	SMI	L8	L19	0.909328
SEAK	L8	L39	0.394292	SMI	L8	L21	0.574123
SEAK	L19	L39	0.673374	SMI	L19	L21	0.023857
SEAK	L21	L39	0.892226	SMI	L8	L31	0.569197
SEAK	L31	L39	0.787572	SMI	L19	L31	0.551695
SEAK	L8	L40	0.778120	SMI	L21	L31	0.416556
SEAK	L19	L40	0.898271	SMI	L8	L39	0.532289
SEAK	L21	L40	0.322900	SMI	L19	L39	0.581500
SEAK	L31	L40	0.610187	SMI	L21	L39	0.558230
SEAK	L39	L40	0.749611	SMI	L31	L39	0.646033
SEAK	L8	L18	0.206285	SMI	L8	L40	0.069747
SEAK	L19	L18	0.627343	SMI	L19	L40	0.333140
SEAK	L21	L18	0.460484	SMI	L21	L40	0.793305
SEAK	L31	L18	0.496474	SMI	L31	L40	0.558006
SEAK	L39	L18	0.642346	SMI	L39	L40	0.910903
SEAK	L40	L18	0.917230	SMI	L8	L18	0.685861
SEAK	L8	L32	0.042855	SMI	L19	L18	0.890014
SEAK	L19	L32	0.657819	SMI	L21	L18	0.982918
SEAK	L21	L32	0.317359	SMI	L31	L18	0.667995
SEAK	L31	L32	0.760290	SMI	L39	L18	0.250792
SEAK	L39	L32	0.262254	SMI	L40	L18	0.330122
SEAK	L40	L32	0.339936	SMI	L8	L32	0.946905
SEAK	L18	L32	0.053360	SMI	L19	L32	0.412348
SEAK	L8	L9	0.170493	SMI	L21	L32	0.062799
SEAK	L19	L9	0.082496	SMI	L31	L32	0.267049
SEAK	L21	L9	0.527254	SMI	L39	L32	0.939288
SEAK	L31	L9	0.529053	SMI	L40	L32	0.864741
SEAK	L39	L9	0.648455	SMI	L18	L32	0.299836
SEAK	L40	L9	0.635789	SMI	L8	L9	0.345737
SEAK	L18	L9	0.814986	SMI	L19	L9	0.811707
SEAK	L32	L9	0.940390	SMI	L21	L9	0.406492
SEAK	L8	L27	0.642224	SMI	L31	L9	0.636454
SEAK	L19	L27	0.011550	SMI	L39	L9	0.610095
SEAK	L21	L27	0.361531	SMI	L40	L9	0.876387

Populations: 9396_PI (Pribilof Island samples collected in 1993 and 1996), 93_SMI (St. Matthew Island samples collected in 1993), SSG (South Shelikhov Gulf), NSG (North Shelikhov Gulf), CSB (Chaunskaya Bay) LD (Little Diomede), PI (Pribilof Islands), SEAK (Southeast Alaska), SMI (St. Matthew Island), and WBS (Western Bering Sea).

Table A-10: Percent chance of detecting multiple paternity in single BKC brood under various relative reproductive contributions of each sire.

Number of Sires	Relative Contribution	% Chance of Detection
2	1:1	98.9
2	9:1	82.7
3	1:1:1	100
3	3:2:1	99.7
overall		> 99.9

Appendix B

Development of polymorphic microsatellite markers for blue king crab

Development of polymorphic microsatellite markers for blue king crab (*Paralithodes platypus*)

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Keywords: *Paralithodes,* Illumina, microsatellite, PAL_FINDER, PCR primers, SSR,

Abstract

We isolated and characterized a total of 23 microsatellite loci from the blue king crab, *Paralithodes platypus.* Loci were screened in 24 individuals from St. Matthew Island. The number of alleles per locus ranged from 4 to 17, observed heterozygosity ranged from 0.050 to 1.000, and the probability of identity values (the probability that two individuals drawn at random from a population will have the same genotype at multiple loci) ranged from 0.015 to 0.339. These new loci will provide tools for examining the genetic population structure of the species throughout its range.

Blue king crab are patchily distributed in the North Pacific Ocean from the Sea of Japan to Southeast Alaska. Surprisingly little is known about the life history and population structure of blue king crab, despite their large commercial value. Commercial blue king crab fisheries in the Bering Sea were worth millions of dollars annually in the 1980s, but crashed and were closed in 1999. The blue king crab commercial fishery near St. Matthew Island reopened in 2009, but the Pribilof Island king crab fishery has remained closed due to low abundance. Efforts to rebuild and manage blue king crab stocks have been hindered by a lack of knowledge about juvenile and adult life stages, mating system, dispersal patterns, and population structure.

Blue king crab are difficult to study in the wild and population genetics studies are one way to gain important information about the species (Palsboll 1999). However, there are currently no published studies on blue king crab genetics, nor have any blue king crab - specific primers been developed. Here, we report 23 new microsatellite markers for blue

king crab, *Paralithodes platypus*, which could be useful in determining genetic population structure.

Total DNA was extracted from one individual of *Paralithodes platypus* by using a standard proteinase K and ammonium acetate precipitation technique for use in isolation of microsatellite loci (Sambrook 2001). An Illumina paired-end shotgun library was prepared by shearing 1 µg of DNA using a Covaris S220 ultrasonicator and following the standard protocol of the Illumina TruSeq DNA Library Kit and using a multiplex identifier adaptor index. Illumina sequencing was conducted on the HiSeq with 100 bp paired-end reads. Five million of the resulting reads were analyzed with the program *PAL_FINDER_v0.02.03* (Castoe et al. 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexanucleotide microsatellites. Once positive reads were identified in *PAL_FINDER_v0.02.03* they were entered into a local installation of the program Primer3 (version 2.0.0) for primer design. To avoid issues with copy number of the primer sequence in the genome, loci for which the primer sequences only occurred one or two times in the 5 million reads were selected. Forty-eight loci of the 19,407 that met this criterion were chosen. One primer from each pair was modified on the 5’ end with an engineered sequence (CAG tag with a sequence of: 5’-CAGTCGGGCGTCATCA-3’) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled. The sequence GTTT was added to primers without the universal CAG tag addition.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04μM tag labeled primer, 0.36μM universal dye-labeled primer, 3.0mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold® Polymerase (Applied Biosystems), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures were used for all loci. Temperature ranges were between 65-55°C (TD65) or 58-48°C (TD58).

Touchdown cycling parameters consisted of an initial denaturation step of 5 min at 95°C followed by 20 cycles of 95°C for 30 s, highest annealing temperature for 30 s (65° or 58° C, depending upon the temperature range) which was decreased 0.5°C per cycle, and 72 °C for 30 s; and 20 cycles of 95°C for 30 s, lowest annealing temperature for 30 s (55° or 48°C depending upon the temperature range), and 72 °C for 30 s. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody et al (2004), except that unlabeled primers started with GTTT. Results were analyzed with Gene Mapper version 3.7 (Applied Biosystems). Twenty three of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of the 23 polymorphic loci in 24 specimens collected from St. Matthew Island, Alaska, USA. Conditions and characteristics of the loci are provided in Table 1. We estimated the number of alleles per locus (*k*), observed and expected

heterozygosity (H_o and H_e), and probability of identity (PI) with GenAlEx v6.4 (Peakall & Smouse 2006). Tests for deviations from Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons one locus showed significant deviation from expectations under HWE and no linkage disequilibrium was detected for any of 253 paired loci comparisons. These new loci will assist in examining the genetic population structure of BKC.

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Table B-1: Details for 23 polymorphic microsatellite loci developed for *Paralithodes platypus*.

Locus	Primer Sequence 5' --> 3'	Repeat motif	Size (bp)	N	K	H_o	H_e	PI	TD
Locus4	F: *GCTCTGTACCATCCACCCG	TCTG	94-110	22	5	0.636	0.588	0.216	65
	R: CAGCTGCCAGACACACAGG								
Locus7	F: *CATAAATATCAAACAACAGGGAGGC	ATCT	160-228	23	7	0.652	0.726	0.124	65
	R: ATGTGTGTGCGCAATATGGG								
Locus8	F: *CCACGAAGTCCTTGACCACG	ATAC	174-232	22	9	0.591	0.572	0.202	65
	R: CAGAGCCCTGAAACCATTACTAGC								
Locus9	F: *CCCTTATTAACGCTTCCATCCC	AGTG	102-130	20	9	0.550	0.668	0.129	65
	R: CAAAGTACAACTGTTAGGAGAGAGGG								
Locus12	F: *CTGTTTCGTCACTGTTAGTAATATCG	ATCT	131-163	24	9	0.708	0.804	0.064	58
	R: GATTGTAATGGCAATCTTGTGG								

Table B-1 continued

Locus	Primer Sequence 5' --> 3'	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Locus14	F: *GAGGGAGATAGCTGGGAGG	AGGT	161-189	23	6	0.696	0.693	0.133	65
	R:CCTTCCAACTTACAGTATTTATCAACC								
Locus15	F: *GATATGTGGTGAGAAAGTTATGTTGG	TCTG	158-218	23	14	0.957	0.905	0.017	65
	R: GGGAAGGACGGAGAATAGGG								
Locus16	F: *GCACACATATTACCTTTCTTCACTACC	AGTG	155-207	24	17	0.958	0.906	0.015	65
	R:AGTGAAATGAACCAGAGCTACG								
Locus17	F: *GCAGGCTCCCTTTCGAGC	ATT	236-345	23	7	0.652	0.705	0.126	65
	R:TTTGGCAATCTTAAAGTGTTTAGGC								
Locus18	F: *GCCACATCACGTAAATGAATAGG	ATCT	131-167	20	8	0.750	0.606	0.180	65
	R:GCTGCTCTCTCCTTGTTGGG								

Table B-1 continued

Locus	Primer Sequence 5' --> 3'	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Locus19	F: *GCGATTACGCTGGAGGTAGG	ATAC	114-134	23	5	0.478	0.450	0.339	65
	R:CACACTGTTCACCTATAATATCCCG								
Locus21	F: *GGCCAGTGATTCATAAACCG	TCTG	117-149	24	8	0.667	0.727	0.115	65
	R:TCTGGTGGGTTTCATTGAGC								
Locus27	F: *GGGCTTCGATTATCGGTTCG	ATCT	92-127	23	6	0.435	0.594	0.244	65
	R:AAGAGTCGCGTGTCAGAGGG								
Locus29	F: *TCCAAAGACACATACAGGACTGG	TCTG	299-355	22	15	0.818	0.857	0.032	65
	R: ACACAAACTATAGCCACTGTCCC								
Locus31	F: *TCGCCTAGGCAGGATATGG	ATAC	174-226	23	10	0.739	0.757	0.081	65
	R: TTTCCCATTTAAATTCCAAAGC								

Table B-1 continued

Locus	Primer Sequence 5' --> 3'	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Locus32	F: *TCTATCCTTCCAGGAATCTGCC	ATCT	138-190	24	10	0.667	0.809	0.058	65
	R:CTGTCGTCATACCTGGCTGC								
Locus35	F: *TCTGTTTGCTTGTCGCTTCC	ATGG	111-151	20	9	1.000	0.805	0.062	58
	R:TTCTCACATTTCCAACCATCC								
Locus37†	F: *TCTTGCTACTATTAGACAATATTATTCAGC	TTCC	214-238	20	4	0.050	0.581	0.236	58
	R:AGGCTGGCAGGAAGTATGG								
Locus39	F:*TGAATTACACGCAGTATTTATATCCACC	ATCT	223-255	23	9	0.652	0.718	0.106	65
	R: TGAGTTAAATAAATTCTGGACAACAAGG								
Locus40	F: *TGCTAATGAAGGAGGCCTGG	AATG	126-142	23	5	0.652	0.522	0.309	65
	R:CACAAGTCCTACACACTTCATTTCC								

* indicates CAG tag (5'- CAGTCGGGCGTCATCA-3') label; † indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections. The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for pcr (see text).